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Turfgrass

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1. INTRODUCTION

Turfgrasses are grasses that provide vegetative ground cover, which is usually mowed routinely. Turfgrasses are used worldwide for lawns of home and office buildings, athletic fields, other recreational facilities, and roadsides. In the United States, there are more than 50 000 000 lawns and 14 000 golf courses and the turfgrass area was estimated in 1990 to be 30 million acres (12 million hectares) (Emmons, 1995). The turfgrass industry is a multibillion dollar a year business. Turfgrasses consist of a remarkably diverse group of species, mostly perennial grasses from the *Poa* family, which are selectively used based on the applications and/or environmental conditions. In general, based on their origins and geographical distributions, turfgrasses can be divided into two groups: cool-season grasses and warm-season grasses. Major cool-season turfgrasses include tall fescue, Kentucky bluegrass, perennial ryegrass, creeping and colonial bentgrasses, and fine fescues. Warm-season grasses used for turf contain bermudagrass, St. Augustinegrass, centipedegrass, Japanese lawngrass, buffalograss, seashore paspalum, and bahiagrass. Most of the cool-season grasses are propagated by seeds while a majority of the warm-season grasses are vegetatively propagated. Many of these species can also be used as forage crops.

Tall fescue (*Festuca arundinacea* Schreb.) is an outcrossing, allohexaploid species, with $2n = 6x = 42$. The genome size of tall fescue is estimated as 7.5×10^9 bp $1C^{-1}$. Tall fescue is of bunch-type growth and primarily spreads by tillering. It is more tolerant to heat and drought among the cool-season grasses and is widely used in the transition zone and the southern regions of the cool season zone. Tall fescue is also an important forage crop (Meyer and Watkins, 2003).

Kentucky bluegrass (*Poa pratensis* L.) is the most widely used turfgrass in the cool-season zone, and spreads with strong rhizomes to produce a dense turf. The reproduction mode and the cytology of Kentucky bluegrass are complicated issues. Kentucky bluegrass is aneuploid and produces seeds both asexually through apomixes and sexually. The level of apomixes among cultivars could vary from 25% to 96%, and the chromosome numbers within the species range from 24 up to 124 (Huff, 2003). The chromosome number among somatic cells within a genotype could vary too. An average chromosome is estimated to be 0.13–0.14 pg (picogram) of DNA (Huff, 2003) or approximately 0.13×10^9 bp/chromosome, and the genome size varies according to the chromosome numbers. A popular cultivar, Midnight, which has the chromosome number of 58–62, was estimated to be approximately 4.1×10^9 bp $1C^{-1}$.

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Perennial ryegrass (*Lolium perenne* L.) is an outcrossing species although forced selfing can be achieved (Thorogood, 2003). It is diploid with $2n = 2x = 14$, and has an estimated genome size of approximately 2.1–2.5 $\times$ 10^9 bp 1C^{-1}. It has a bunch-type growth habit, and is often a choice for athletic fields for its good wearing resistance. Perennial ryegrass is also widely used for winter overseeding of warm-season grass turf in the subtropical zone partially because of its rapid establishment.

Creeping bentgrass (*Agrostis stolonifera* L. or *Agrostis palustris* Huds.) has a fine texture and tolerance to mowing as low as 3 mm. Requiring high maintenance, the stoloniferous growth habit of creeping bentgrass provides carpetlike, soft, dense sod, and is mostly used for high quality turf on golf course putting greens, tees, and fairways. The outcrossing creeping bentgrass is allotetraploid with $2n = 4x = 28$ (Warnke, 2003) and has an estimated genome size of 2.7 $\times$ 10^9 bp 1C^{-1}.

Colonial bentgrass (*Agrostis capillaris* L. or *Agrostis tenuis* Sibth.) is mostly used as a fairway grass and for erosion control. Outcrossing is the primary mode of sexual reproduction. The species is allotetraploid with $2n = 4x = 28$ (Ruemmele, 2003) with an estimated genome size of 2.8 $\times$ 10^9 bp 1C^{-1} (based on Bonos *et al.*, 2002).

Fine fescues are a group of fine-leaved *Festuca* species, containing two main species complexes: the red fescue (*F. rubra*) complex and the *F. ovina* complex (Ruemmele *et al.*, 2003). Being important turfgrasses in Europe and the United States, fine fescues are mostly used as turf in dry, shady, and low-fertile soils. Fine fescues are primarily open-pollinated species. The chromosome number of *F. rubra* complex species is $2n = 28$, 42, 56, and 70 with most of the plants being hexa- or octoploids, whereas *F. ovina* complex is $2n = 14$, 28, or 42. A hexaploid hard fescue (*Festuca trachyphyllas* or *Festuca longifolia*) cultivar Aurora in this complex has an estimated genome of 6.1 $\times$ 10^9 bp 1C^{-1}.

Turf-type bermudagrass mainly includes common (or seeded) bermudagrass (*Cynodon dactylon* (L.) Pers. var. *dactylon*), a fertile tetraploid ($2n = 4x = 36$) species, and the interspecific hybrid (*C. dactylon* $\times$ *Cynodon transvaalensis* Burtt-Davy), a sterile triploid ($2n = 3x = 27$) (Taliaferro, 2003). The genome sizes of the two are estimated to be 0.94 $\times$ 10^9 bp 1C^{-1}, and 0.66–0.78 $\times$ 10^9 bp 1C^{-1}, respectively. Hexaploid ($2n = 6x = 54$) of *C. dactylon* was also reported (Hanna *et al.*, 1990). Bermudagrass is the most important warm-season turfgrass found from temperate to tropical regions and forms good quality turf. The vegetatively propagated hybrid cultivars Tifgreen, Tifway, and the new TifEagle are widely used in golf greens and fairways in the warm-season zone. Common bermudagrass is outcrossing and propagated by seeds.

St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) is a major turfgrass species in subtropical and tropical regions. In 2001, 70% of the lawns grown in Florida were St. Augustinegrass. The grass spreads by branching stolons and, in general, has coarse leaves. St. Augustinegrass is vegetatively propagated. Although artificial crossing can be readily performed, attempts for seed propagation were not successful. St. Augustinegrass is often $2n = 2x = 18$, although sterile triploid ($2n = 3x = 27$) and aneuploid were also reported (Busey, 2003). The genome size of a diploid cultivar, Raleigh, was estimated to be 0.52 $\times$ 10^9 bp 1C^{-1}.

Centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) is a turfgrass species requiring only low maintenance and still provides acceptable turf quality (Hanna and Liu, 2003). It spreads by stolons, and is used in lawns and roadsides. The grass is seed propagated with $2n = 2x = 18$ chromosomes. The genome size of centipedegrass is 0.8 $\times$ 10^9 bp 1C^{-1}.

Japanese lawngrass (*Zoysiagrass japonica*) is a turfgrass used for lawns, golf fairways, and athletic fields. It spreads by rhizomes and stolons, and can be either vegetatively or seed propagated. The chromosome number of the species is mostly reported as tetraploid with $2n = 4x = 40$ although diploidy was also recorded (Engelke and Anderson, 2003). The tetraploid genome size was estimated as 0.42 $\times$ 10^9 bp 1C^{-1}.

Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm) is a native North American grass used extensively as a turfgrass. Requiring minimum care, the grass spreads by branching stolons, is drought tolerant, and has excellent erosion control. Being a warm-season grass, buffalograss is also quite cold tolerant. Buffalograss is a dioecious species with separate male and female flowers that often occur in separate plants. The grass is mostly propagated vegetatively, and sometimes

by seeding (Riordan and Browning, 2003). Di-, tetra-, and hexaploidy ($2n = 2x$, $4x$, $6x = 20$, 40, 60) have been reported in buffalograss. Cultivar 609 is a tetraploid with a genome size of 0.77×10^9 bp $1C^{-1}$.

Seashore paspalum (*Paspalum vaginatum* Swartz) is a very unique, good-quality turfgrass species. It has high tolerance to salinity, drought, waterlogging, and low light intensity; adapts to a wide range of pH (3.6 to 10.2); and requires low maintenance (Duncan, 2003). It has been used as a salt-tolerant turfgrass in golf courses, sports fields, and for general landscaping, as well as utility turf for erosion control at coastal regions. The grass spreads by stolons and rhizomes, is outcrossing, but propagated vegetatively. Seashore paspalum is predominantly diploid with $2n = 2x = 20$ although tetra- and hexaploidy were reported. The genome size of the grass is not determined.

A chapter in the series is dedicated to bahiagrass (*Paspalum notatum* Flugge). Thus, the species would not be further discussed in this chapter.

Unless otherwise specified, the genome sizes were estimated based on the nuclear DNA content of individual species reported by Arumuganathan *et al.*, 1999, and the information that 1 pg DNA $\approx 0.965 \times 10^9$ bp (Arumuganathan and Earle, 1991).

Methodical turfgrass breeding probably started around the turn of the 20th century, and was substantially intensified in the past few decades (Casler and Duncan, 2003). The passage of the Plant Variety Protection (PVP) Act in the 1970s by the US Congress greatly encouraged and inspired the private businesses in the United States to breed new crop cultivars, including those of turfgrasses. A more uniform, dense turf with finer and greener leaves is a major target trait for improvement in turfgrass breeding. In addition, it should be realized that, although turfgrasses arose from natural habitats, the environments for them to be used as turf are "human-defined", and often much more stressful than their original habitats, including many biotic and abiotic stress factors (Casler and Duncan, 2003). Thus, main breeding efforts are also toward resistance to (mostly fungal) diseases and insects, and tolerance to drought, extreme temperatures, shade, and in some cases, mowing. Recurrent selection of a population resulted from intercrossing of several individual parent clones, when possible, is probably the mostly used approach for new cultivar development in turfgrass species. Interspecific hybridization and irradiation mutagenesis also play important roles in turfgrass breeding (Casler and Duncan, 2003). Transgenic technology was first applied to turfgrasses in the early 1990s, and so far transgenic plants have been successfully generated in nearly a dozen turfgrass species. Transgenic technology can break the barriers of gene exchange between species, and even kingdoms. It also can up- or down-regulate an individual endogenous gene for improvement of trait(s). For example, an herbicide or disease resistance gene of bacterial origin is readily introduced into a turfgrass species using the technology while no such gene can be found in the germplasm pool of the particular species. However, because of the perennial growth habit of turfgrasses and the outcrossing nature of most of the turfgrass species, the risk of transgene escape is a major concern in releasing transgenic turfgrass cultivars. Since tissue culture is usually a prerequisite for successful genetic transformation of turfgrasses, we will examine the achievements in tissue culture of various turfgrass species first in the chapter, which will be followed by the reported successes in transformation technologies in turfgrasses, improved traits by genetic transformation, regulatory issues on release of transgenic turfgrass, and future perspectives of turfgrass improvement through biotechnology.

2. GENETIC TRANSFORMATION OF TURFGRASSES

2.1 Tissue Culture Studies of Turfgrasses

With few exceptions (Clough and Bent, 1998), almost all plant transformation has to go through a tissue culture stage to deliver genes to embryogenic cells and to eventually regenerate transgenic plants from those cells. Thus, optimization of tissue culture responses of a plant species, especially improvement of regeneration ability, often plays a key role in the success of transformation efforts. Although not all the turfgrass species mentioned above have been transformed, tissue culture studies have been reported for each of them. In addition to obtaining transgenic plants, tissue culture also serves purposes such as rapid propagation of plants or creation of somaclonal variation

for breeding efforts (Brown and Thorpe, 1995). Tissue culture responses are generally affected by genotype and explant type of the species, the composition and supplements of the culture media, the phytohormones added in the media, and, sometimes, other factors. In the following description, we try to highlight the major approaches and the accomplishments in tissue culture found in the literature for each major turfgrass species, so the readers can have an historical view of the progresses in the field.

Krans (1981) obtained calli of tall fescue by culturing mature caryopses at 5 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), and plantlets from regeneration medium containing 0.01 mg l^{-1} 2,4-D and 0.2 mg l^{-1} kinetin. Lowe and Conger (1979) cultured mature embryos of tall fescue (cv. Kentucky 31) on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 9 mg l^{-1} 2,4-D and observed almost 100% callus induction. About 18% calli regenerated shoots and the regeneration ability declined along with subculture on 5 mg l^{-1} 2,4-D. Dale and Dalton (1983) cultured immature inflorescences (1–20 mm in length) in MS medium containing 2 mg l^{-1} 2,4-D with or without 0.2 mg l^{-1} 6-benzylaminopurine (BAP), and obtained 10.2 plantlets per inflorescence. The shoots could directly form from floret primordia or through callus. Eizenga and Dahleen (1990) studied culture responses of partially emerged inflorescences of various genotypes within cv. Kentucky 31 and observed significant variations among genotypes in callus induction and plant regeneration. The explants were cultured on Schenk and Hildebrandt (SH) (Schenk and Hildebrandt, 1972) medium with various auxins. Callus production was significantly higher when 2,4-D was used as the auxin source over para-chlorophenoxyacetic acid (pCPA) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Bai and Qu (2000) compared tissue culture responses of mature caryopses among 25 turf-type tall fescue cultivars on MS medium containing 9 mg l^{-1} 2,4-D, and observed a wide range of callus induction rates (from 4.4% to 51.9%). The callus regeneration rates were from 16.7% to 58.8% on MS regeneration medium containing 2.5 mg l^{-1} BAP. The overall plant regeneration rates (regeneration callus over explants cultured) varied from 1% to 22%, reflecting substantial differences among cultivars. In another report, the same group studied effects of medium supplements on culture responses of an elite turf-type cultivar, Coronado (Bai and Qu, 2001a). They found inclusion of BAP (0.1 mg l^{-1}) in callus induction medium significantly improved callus regeneration for explants of immature embryo and mature caryopses. Casein hydrolysate (CH), L-proline, and myo-inositol improved callus induction of immature embryos but not mature caryopsis. These supplements had no effect on callus regeneration. Longitudinally slicing mature caryopses into two halves improved their callus induction by three- to sixfold. The optimal supplement combination for the sliced caryopses was 5 or 9 mg l^{-1} 2,4-D and 0.1 mg l^{-1} BAP, which yielded about 30% overall plant regeneration. Dalton (1988) developed suspension cultures in MS-based medium from embryogenic calli induced from chopped mature embryos of tall fescue. Up to 0.4% of protoplasts isolated from the suspension culture formed colonies when cultured in glucose-containing medium, and more than 100 colonies formed green shoots. By using agarose bead method and nurse culture, Takamizo *et al.* (1990) also obtained green plants from protoplasts isolated from a suspension line that was derived from a young leaf-base culture. In another direction, Kasperbauer *et al.* (1980) obtained anther-derived haploid plants from culture of panicle sections containing several florets after a cold temperature pretreatment. Later on, doubled haploid plants were recovered after subculturing the haploid calli for 15 weeks to reach an "aged" stage and then transferred to a regeneration medium (Kasperbauer and Eizenga, 1985). Somaclonal variation among the regenerated tall fescue plants was observed in traits such as total biomass, seed yield, and leaf shape (Roylance *et al.*, 1994; Bai, 2001). Garcia *et al.* (1994) reported great alteration in flowering response among regenerated plants: some plants flowered without vernalization treatment, and a plant did not flower after 2 years with vernalization. Chromosome number usually did not change but chromosome aberrations were observed. Since maintenance of suspension culture is labor intensive and the regeneration ability of the suspension lines usually declines along with time, Wang *et al.* (1994) explored conditions of cryopreservation of the suspension cells, and found that high-osmotic preculture and cryoprotectant containing 10% dimethyl sulfoxide (DMSO) and

0.5 M sorbitol were the most suitable for storage of tall fescue suspension cells in liquid nitrogen. Up to 71% of the cells could regrow after cryopreservation, and embryogenic suspensions were re-established. Bai (2001) found that a cryoprotectant recipe used for rice and barley, which contains 0.5 M DMSO, 0.5 M glycerol and 1 M sucrose, was the best among the tested, and did not require osmotic preculture treatment. The cells, after being stored for 30 days in liquid nitrogen, increased by 65% in fresh weight after 4 weeks of culture on a solid medium.

Krans (1981) reported callus induction and root formation of Kentucky bluegrass. McDonnell and Conger (1984) used mature embryos as explants and a modified SH medium, and observed that 20 μM dicamba or 60 μM picloram was most effective in callus growth and in inhibiting leaf growth. The regeneration rates were very low (0–3.1%) but were improved by culturing at a low temperature (15 °C), and further enhanced (up to 18%) by a week of cold treatment (4 °C) before transferring to the regeneration medium. Boyd and Dale (1986) used mature embryo culture to evaluate 50 cultivars for their callus induction and plantlet regeneration ability, and found great variations among the cultivars. The authors considered that MS basal medium supplemented with 0.2 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BAP, 100 mg l^{-1} CH, and 25 g l^{-1} sucrose was good for both callus growth and plantlet regeneration. Wu and Jampates (1986) obtained plantlets from calli induced from shoot tips. Van Der Valk *et al.* (1989) studied tissue culture responses of 15 cultivars, and found that calli from immature inflorescences of 14 cultivars regenerated plantlets and the regeneration rates were often over 50%, whereas regeneration of calli from mature caryopses were very low: plantlets were recovered from only 3 cultivars with regeneration frequencies below 3%. Van Ark *et al.* (1991) found regeneration frequency was doubled when Gelrite was used as a gelling agent over agar, and abscisic acid (ABA) enhanced somatic embryogenesis although regeneration rate was not affected. In 1995, two groups reported high frequency of regeneration from mature caryopsis-induced calli by including BAP in the callus-induction medium (Griffin and Dibble, 1995; Van Der Valk *et al.*, 1995). Ke and Lee (1996) investigated culture responses of tissues from young seedlings, and observed that calli induced from coleoptiles had highest regeneration frequency (32%) when the regeneration medium contained 0.2 mg l^{-1} picloram and 0.01 mg l^{-1} α-naphthalene acetic acid (NAA). Salehi and Khosh-Khui (2005) observed that the callus induction rate from mature caryopses was 100% when 40 μM (8.85 mg l^{-1}) 2,4-D was added to the induction medium and 100% regeneration was achieved when 60 μM 2,4-D (13.3 mg l^{-1}) and 12.5 μM (2.8 mg l^{-1}) of BAP was used in the regeneration medium. Hu *et al.* (2006) cultured shoot apices of seedlings in a medium containing low 2,4-D (0.9 μM or 0.2 mg l^{-1}) and 8.9 μM (2 mg l^{-1}) BAP to induce multiple shoot clumps, which came from meristematic cell clumps originated from embryonic axis, especially the hypocotyls in the culture. In addition, green plantlet regeneration from embryogenic suspension cell culture (Nielsen and Knudsen, 1993) and protoplast (Nielsen *et al.*, 1993) were reported. The suspension lines were derived from calli induced from mature caryopses or mature embryos of cv. Geronimo. Using the agarose-embedding technique, the protoplast plating efficiencies were demonstrated to vary from 0.004% to 1.5%, and 0.4% to 2.7% of the protoplast-derived microcolonies were able to regenerate.

Krans (1981) also first reported perennial ryegrass plants regenerated through tissue culture using an approach similar to he did with tall fescue. With mature caryopses as explant, Torello and Symington (1984) found subculture of calli on medium containing 5 mg l^{-1} of 2,4-D was preferred over 10 mg l^{-1}, with 0.1 mg l^{-1} BAP in regeneration medium being optimal for shoot regeneration. The same group (Zaghmout and Torello, 1992a) reported advantages to include cefotaxime (60–200 mg l^{-1}) in culture medium for improved green plantlet formation. The authors observed that "cefotaxime reduced the inhibitory effect of 2,4-D on precocious germination of somatic embryos." Dale and Dalton (1983) cultured immature inflorescences (1–20 mm in length) in MS medium, and found 50% more shoots were formed directly or through callus when the medium containing 2 mg l^{-1} 2,4-D was supplemented with 0.2 mg l^{-1} BAP and 100 mg l^{-1} casein hydrolysate (CH). In mature caryopsis culture, Bradley *et al.* (2001) observed substantial variations in callus induction (2.3–21%) and regeneration (0–58%) among 13 elite turf-type

cultivars. Slicing of the caryopsis longitudinally increased callus induction rate by more than fivefold when 2,4-D level was 2 mg l^{-1}. Inclusion of BAP (0.5 mg l^{-1}) in callus induction and subculture medium enhanced its regeneration rate by more than threefold. Salehi and Khosh-Khui (2005) observed 98% callus induction rate from mature caryopses (cv. Barbel) when 2,4-D concentration in the callus induction medium was as high as 150 μM (33.3 mg l^{-1}). They obtained 100% callus regeneration when the regeneration medium contained 60 μM 2,4-D (13.3 mg l^{-1}) and 7.5 μM (1.7 mg l^{-1}) BAP. In suspension culture, Olesen *et al.* (1996) examined suspensions from 21 commercial varieties and could regenerate green plantlets from 18 of them. In a multifactorial experiment to optimize suspension culture conditions, Altpeter and Posselt (2000) observed that replacement of sucrose by maltose, dicamba by 2,4-D (5 mg l^{-1}), and a stepwise increase of BAP to 0.25 mg l^{-1} enhanced green shoot regeneration ability of the suspensions, but addition of CH did not. Using this medium, together with the best-performed genotype, L6, 488 green plantlets were regenerated from per gram fresh weight suspensions. Dalton (1988) reported recovery of green plantlets from protoplast culture of perennial ryegrass without agarose embedding and nurse culture. Creemers-Molenaar *et al.* (1989) observed that protoplast from "nonmorphogenic" suspensions had higher plating rate than the ones from "morphogenic" suspensions, and also recovered green plantlets from protoplast culture. The same group (Creemers-Molenaar *et al.*, 1992) reported that conditioned medium was "indispensable" for plating of protoplasts from younger, regenerable suspensions. O-acetylsalicylic acid and some antioxidants also helped improve plating efficiency. Wang *et al.* (1993) established single genotype-based embryogenic suspension lines and cultured protoplasts using agarose bead and nurse cells. Protoplasts from cryopreserved suspensions performed similarly to the ones isolated from the fresh suspensions and dozens of green plantlets were regenerated. Moreover, Olesen *et al.* (1988) reported successful anther culture of perennial ryegrass after cold pretreatment without nurse culture, and found that recovery of green plantlets were mostly from 2 genotypes. Perezvicente *et al.* (1993) cultured vegetative apices (0.05–0.3 mm) consisting of the meristematic dome and 1–4 leaf primordia in MS medium containing 0.01 mg l^{-1} 2,4-D and 0.2 mg l^{-1} kinetin under dim light condition. About 99% of the explants formed plantlets. Plants regenerated from suspension cell cultures of perennial ryegrass were compared to the seed-grown plants in the field (Stadelmann *et al.*, 1998). Quite often, the regenerated plants showed a delay in inflorescence emergence, a reduced seed yield, and other morphological variations. Sometimes, alterations in DNA sequences were detected by random amplified polymorphic DNA markers. Plants with similar or superior performance to the seed-grown plants were also observed.

Successful tissue culture of creeping bentgrass was first reported by Krans (1981) using caryopses as explants. The greatest callus induction came from cultures in medium containing 1 mg l^{-1} 2,4-D under light, or containing 1 mg l^{-1} 2,4-D and 0.01 mg l^{-1} kinetin in the dark. Calli cultured on 1 mg l^{-1} 2,4-D maintained good regeneration ability and the best regeneration medium contained 0.1 or 1 mg l^{-1} kinetin. Using a 24-week-old stock callus, Blanche *et al.* (1986) observed that callus growth was twice greater after being cultured for 12 or 24 days in suspension, whereas suspension culture greatly reduced plantlet formation. They also determined that the callus growth rate and regeneration frequency were correlated to the size of callus aggregates and the plating density. Zhong *et al.* (1991) evaluated various plant growth regulators on embryogenesis of creeping bentgrass caryopses culture, and observed that MS basal medium containing 30 μM (6.63 mg l^{-1}) dicamba and 2.25 μM (0.5 mg l^{-1}) BAP was optimal, in which 91% calli were embryogenic and over 80% of the somatic embryos formed plantlets after transferring to the regeneration medium. Terakawa *et al.* (1992) reported microcolony formation and 0.36% plating efficiency from protoplasts of creeping bentgrass, cv. Penncross, cultured in a conditioned medium. Thirty percent of the microcolonies formed green plantlets. Lee *et al.* (1996) developed an efficient system for protoplast isolation, culture and regeneration from 7 cultivars. Using feeder cells, the plating frequencies ranged from 0.05% to 0.32%.

Torello *et al.* (1984) observed different culture responses of mature caryopses of two cultivars in red fescue, and investigated the effects of auxin sources on callus growth. They found 20 μM (4.4 mg l^{-1}) 2,4-D was the best in callus

growth, and used 0.1 mg l^{-1} BAP for regeneration medium. In general, regeneration ability of a long-term callus culture declined. The same group found that addition of activated charcoal to the callus maintenance medium prior to callus transferring to the regeneration medium significantly enhanced the regeneration ability of calli cultured for over 5 years (Zaghmout and Torello, 1988). They believed that the activated charcoal could adsorb toxic levels of 2,4-D and other morphogenesis inhibitors. These authors later developed suspension cultures using a modified MS medium containing 4 mg l^{-1} 2,4-D and 3 g l^{-1} CH with the latter enhancing culture growth significantly. Removal of fast-growing, nonembryogenic cell clusters before initiation of suspensions was thought to be critical, and green plantlets were regenerated from the suspensions (Zaghmout and Torello, 1989). In addition, the same authors found that elevating sucrose level from 60 mM up to 120 and 180 mM in suspension as well as regeneration media helped restore regeneration potential of long-term suspension cultures, and increased green plantlet formation (Zaghmout and Torello, 1992b). Protoplasts were isolated from the embryogenic suspension cells and plated in agarose squares surrounded by nurse cells. The maximum plating efficiency was 1%. The microcolonies produced embryos, which germinated to form plantlets in regeneration medium (Zaghmout and Torello, 1990). Altpeter and Xu (2000) tested tissue culture responses of mature embryos from 7 turf-type red fescue cultivars, observed callus induction rates ranged from 57% to 78.4% in a medium containing 5 mg l^{-1} 2,4-D, and found significant difference in callus regeneration ability (from 0% to 22%) on a medium containing 0.1 mg l^{-1} 2,4-D and 0.1 mg l^{-1} BAP. Recently, Salehi and Khosh-Khui (2005) reported that very high 2,4-D concentration (200 μM or 44.4 mg l^{-1}) enhanced callus induction rate (78%) of red fescue (cv. Shadow), and a combination of 60 μM (13.3 mg l^{-1}) of 2,4-D and 12.5 μM (2.8 mg l^{-1}) of BAP resulted in the highest callus regeneration rate (34%) of red fescue.

The only report on colonial bentgrass tissue culture (Wang *et al.*, 2002) evaluated 14 MS-based culture media containing various phytohormones and other supplements for mature caryopses culture of cv. Exeter. Eleven media that contained 2,4-D as an auxin had high callus induction rates (98–100%). In general, embryogenic callus induction was low. Although the best callus induction medium was the MS medium containing 5 mg l^{-1} 2,4-D and 500 mg l^{-1} L-proline, which had 100% callus induction and yielded 19% "embryogenic calli", they regenerated poorly. On the other hand, the media containing dicamba (6.63 mg l^{-1}), BAP (0.5–2 mg l^{-1}), and CH (500 mg l^{-1}) had low callus induction rates (20–37%), but relatively high embryogenic callus frequencies (6–10%), and the embryogenic calli were more regenerable. CH by itself seemed to promote callus regeneration. Media containing 2,4-D (2–5 mg l^{-1}) and CH had 5–7% embryogenic callus formation and high regeneration rates from those calli.

In warm-season turfgrasses, callus induction of common bermudagrass was first reported by Krans (1981). Ahn *et al.* (1985) observed that calli had significantly more fresh weight when growing on N6 medium (Chu *et al.*, 1975) over the MS medium. The best culture conditions in the report were immature inflorescence of 0.5 cm in length on N6 medium containing 1 mg l^{-1} 2,4-D and 60 g l^{-1} sucrose, from which 84% of calli were embryogenic. Green plantlets were obtained from hormone-free N6 medium and, subsequently, grown to maturity. It seemed the explant type and age played an important role in recovering regenerated plants since no plantlets were regenerated when nodes, root tips, young leaves, mature caryopses or inflorescences longer than 1.5 cm were used as explants. A follow-up report from the same group demonstrated genotype dependence of the regeneration ability of the induced calli. In addition, a regenerable suspension cell culture was established (Ahn *et al.*, 1987). Artunduaga *et al.* (1988) investigated 2,4-D concentrations required by different genotypes of common bermudagrass for best embryogenic callus formation from immature inflorescence. While all the induction medium (a half-strength MS medium) also contained 1 mg l^{-1} indole-3-acetic acid (IAA), the authors found 1 mg l^{-1} 2,4-D was optimal for embryogenic callus formation from an accession while 3 mg l^{-1} was more suitable for two other accessions. Plantlets were regenerated from two out of the three accessions on a half-strength MS medium supplemented with 0.5 mg l^{-1} 2,4-D and 1 mg l^{-1} zeatin although about 90% of the plantlets from an accession were

albino. In another correspondence (Artunduaga *et al.*, 1989), the same group studied 2,4-D concentration and supplement of CH on culture responses of immature inflorescence of *C. dactylon* cv. Zebra. The maximum fresh weight and embryogenic callus induction came from the medium containing 3 mg l^{-1} 2,4-D and 200 mg l^{-1} CH. The albino plants were as high as 31% of the regenerated plants. Chaudhury and Qu (2000) observed that inclusion of 1 mg l^{-1} 2,4-D and very low concentration of BAP (0.01 mg l^{-1}) in the callus induction/subculture medium substantially improved the regeneration ability of the calli from common bermudagrass cv. Savannah and hybrid cv. Tifgreen. The effect was more obvious when younger immature inflorescences (<0.75 cm) were used as explants. All 96 plantlets obtained were green and morphologically normal. Scanning electron microscopy (SEM) revealed embryogenesis was the major pathway for plantlet regeneration in bermudagrass. Li and Qu (2002) found that supplement of ABA (2–5 mg l^{-1}) to the above BAP-containing medium further improved formation of somatic embryo clusters (SEC), and tracked the whole process of SEC formation microscopically over a period of 70 days. In addition, secondary somatic embryogenesis was observed in this species. Moreover, they found that bermudagrass plantlet regeneration was greatly enhanced by addition of GA_3 in the regeneration medium. Li and Qu (2004) further developed highly-regenerable callus lines, including suspension lines, by adjusting BAP level in the medium and carefully selecting calli. Salehi and Khosh-Khui (2005) tested a wide range of 2,4-D concentrations in MS callus induction medium for mature caryopsis culture of common bermudagrass (the cultivar was only identified as "California origin") and BAP concentrations in regeneration medium, and found that 40 μM (8.85 mg l^{-1}) 2,4-D had 100% callus induction frequency, and 60 μM 2,4-D (13.3 mg l^{-1}) and 7.5 μM (1.7 mg l^{-1}) BAP led to 100% callus regeneration with 3.2 plantlets per piece of callus. The widely used hybrid bermudagrass cultivars, Tifgreen (Burton, 1964), Tifway (Burton, 1966), TifEagle (Hanna and Elsner, 1999), and TifSport (Hanna *et al.*, 1997), although all being *C. dactylon* × *C. transvaalensis*, seem to be very divergent in their responses to tissue culture. Goldman *et al.* (2004b) reported 36% embryogenic callus induction rate from nodes of TifEagle plants using an MS medium containing 4.5 μM 2,4-D, 0.044 μM BAP, and 40 g l^{-1} (instead of the routine 30 g l^{-1}) sucrose. In contrary, no embryogenic calli were produced from nodes of TifSport. The best culture of TifSport using very young immature inflorescence yielded 16% of embryogenic calli on the same medium. A great range of somaclonal variation, in terms of plant height, leaf width, leaf length, and number of stolons, was observed from regenerated plants of TifEagle, but not from TifSport. Tifway, on the other hand, may be the most recalcitrant cultivar among the four, with tissue culture responses more similar to a parent, *C. transvaalensis* (Chaudhury and Qu, unpublished data). Although calli were ready to be induced from vegetative tissues (nodes, internodes, and young leaves), and were not prone to browning, they were not regenerable. Severe browning, caused by secretion of phenolic compounds, was encountered when immature inflorescences were cultured, which resulted in little callus induction. Qu and Chaudhury (2001) overcame the problem by treating the inflorescence segments in liquid MS medium containing 0.2% L-ascorbic acid, an antioxidant, followed by culture on an MS callus induction medium supplemented with 6 g l^{-1} polyvinylpolypyrrolidone (PVPP), which is a polyphenol adsorbent. By doing so, calli were induced from over 50% of immature inflorescences smaller than 1.5 cm, and green plantlets were regenerated.

St. Augustinegrass tissue culture was first reported by Kuo and Smith (1993). Immature embryos isolated from inflorescences, 10–14 days after emergence from the sheath, of cv. Texas Common were cultured on MS medium supplemented with various concentration of 2, 4-D for callus induction. Calli induced on medium containing 1 mg l^{-1} 2,4-D grew faster than the calli on 5 mg l^{-1} 2,4-D medium, whereas no calli were induced on medium having 10 mg l^{-1} 2, 4-D. Four weeks later, the calli were subcultured on MS medium containing 0.5 mg l^{-1} 2,4-D and 0.25 mg l^{-1} kinetin for another 4 weeks before they were transferred to the MS medium supplemented with 0.25 mg l^{-1} 2,4-D and 0.5 mg l^{-1} kinetin. After 1–2 weeks, somatic embryos became obvious and shootlike structures were observed. The calli were then transferred to the hormone-free, half-strength MS medium for further growth of the plantlets. Approximately 33% of the induced calli were able

to regenerate plantlets. The authors considered it was critical to have timely transfer of callus at each stage for successful regeneration. Li *et al.* (2006) examined callus induction of 11 types of explants of St. Augustinegrass (cv. Raleigh) and the effect of BAP in callus induction medium. The explants were cultured on MS medium supplemented with 1 mg l^{-1} 2,4-D and various concentrations of BAP. Although they observed that five types of explants had high callus induction frequencies (71–100%), only calli from three kinds of explants were embryogenic and can regenerate plantlets. They are immature embryos harvested at various stages (3 days and 7–14 days) and young shoot base. However, the callus induction of the 3 days immature embryos was below 20%, and the regeneration rate of calli induced from young shoot base was under 10%. In general, addition of BAP in callus induction medium did not affect callus induction but significantly improved callus regeneration ability. The best combination the authors found were the use of 7–14 days immature embryo as explant and a callus induction medium (with 1 mg l^{-1} 2,4-D) supplemented with 0.5 mg l^{-1} BAP, which had 97.7% callus induction frequency and 47.6% regeneration rate. SEM study revealed details of somatic embryogenesis in the species. Approximately 8000 plants were regenerated in a breeding effort to induce somaclonal variations (Li *et al.*, submitted).

Tissue culture of Japanese lawngrass was first reported in 1989 (Alkhayri *et al.*, 1989; Asano, 1989). In Asano's report, calli were induced from 4-day-old seedlings cultured on a modified N6 medium containing various concentration of 2, 4-D or picloram. More fresh weight of callus was recovered from picloram-containing medium. Only 0.3% of calli developed embryogenic sectors, from which green plantlets were obtained on a hormone-free, half-strength MS medium. No obvious morphological variations were observed from the recovered plants. Protoplasts were isolated from callus lines and cultured in the same modified N6 medium supplemented with 5 mg l^{-1} picloram. Plating efficiency was about 20–30%, but no regeneration was observed. Alkhayri *et al.* (1989) had somewhat different observation. They cultured mature embryos separated from caryopses on MS or N6 medium with various 2,4-D concentrations. Over 90% of the explants produced calli in all treatments. The best combination was MS medium with 1 mg l^{-1} 2,4-D; the calli had more fresh weight and higher regeneration frequency (42–59%) after transferred to 2,4-D-free regeneration medium. Calli from N6 medium did not regenerate. Asano *et al.* (1996) found that cytokinin, especially BAP and thidiazuron (TDZ), and thiamine HCl were essential for embryogenesis. In their presence, supplement of riboflavin or α-ketoglutaric acid, a key metabolic intermediate of TCA cycle, further enhanced formation of embryogenic calli. Inokuma *et al.* (1996) observed that LS medium (Linsmaier and Skoog, 1965) had been superior over MS or N6 medium in inducing embryogenic calli in this species. Protoplasts were isolated from the suspensions and the best plating efficiency was 0.15% in medium solidified with agarose. Green plantlets were recovered from the protoplast culture.

In vitro regeneration of buffalograss was first reported in 1997 (Fei *et al.*, 1997). Female immature inflorescences of cv. 609 were cultured in MS medium containing a combination of 2, 4-D (1–4 mg l^{-1}) and BAP (0–0.4 mg l^{-1}). Seventeen percent calli from medium containing 2,4-D only (1–3 mg l^{-1}) regenerated shoots. Interestingly, no calli from BAP-containing medium regenerated. The tissue culture responses depended on genotype and the season when the inflorescences were harvested. Later on, the same group reported that inclusion of silver nitrate (10 mg l^{-1}) in the callus induction medium significantly improved embryogenic callus formation and subsequent regeneration for cultures of immature inflorescences from male plants of two genotypes but not the ones from female plants. The stimulatory effects were environment and genotype dependent (Fei *et al.*, 2000). In another correspondence (Fei *et al.*, 2002), the authors reported that lowering 2, 4-D concentration and addition of BAP enhanced shoot regeneration for 2-month-old calli induced on a medium containing 9 μM (2 mg l^{-1}) 2,4-D from male immature inflorescences of cv. Texoka. The best regeneration medium contained 2.25 μM (0.5 mg l^{-1}) 2,4-D and 0.44 μM (0.1 mg l^{-1}) of BAP, on which 36.4% embryogenic calli regenerated shoots. It was also found that gelling agent Gelrite significantly improved somatic embryo formation over agar. However, these effects also seemed to be genotype dependent since no such enhancement was observed for immature inflorescences collected

from female plants of genotype 315. In addition, embryogenic calli were obtained from leaf base and leaf segments of seedlings from germinating caryopsis of cv. Cody. The induction frequency was relatively low (around 10%) and depended on seedling age and the concentration of 2,4-D in the induction medium. Plantlets were recovered from the embryogenic calli (Fei *et al.*, 2001).

Seashore paspalum is a recently promoted turfgrass species and its tissue culture was not reported until 1997. Cardona and Duncan (1997) used immature inflorescences as an explant from 9 ecotypes of seashore paspalum. The explants were cultured on a medium consisting of the MS basal salts and B5 vitamins. In a factorial experiment designed to test the effects of growth regulators 2,4-D and BAP, it was observed that the effect of BAP or the interaction between BAP and 2,4-D was not significant in callus induction efficiency whereas the effect of 2,4-D was. Responses to 2,4-D concentrations varied among the ecotypes tested. However, a low concentration of 2,4-D (1 to 2 mg l^{-1}) was recommended for embryogenic callus formation. The callus first emerged about a week after culture initiation while "precocious" somatic embryos were observed 3 weeks later. Callus induction frequency varied from 0% up to 55%. Five-month-old calli were cultured on a half-strength MS medium containing different combinations of BAP and NAA for regeneration, and numerous green plantlets were recovered from all the ecotypes. Although the optimized combination of BAP and NAA varied among the ecotypes, the combination of 1 mg l^{-1} BAP with NAA between 0.5 and 2 mg l^{-1} often had the highest regeneration frequency. Ecotypes HI-1, Mauna Key, and PI299042 were among the best in regeneration ability.

Centipedegrass tissue culture was first reported by Krans' lab (Krans, 1981; Krans and Blanche, 1985). One mg l^{-1} 2,4-D or 100 mg l^{-1} IAA was determined to be the optimal concentration for callus induction on MS medium based on fresh callus weight. Fresh callus weight from a single explant was greater using immature inflorescence than mature caryopses. When the calli were transferred to regeneration medium, either hormone free or containing 0.5 mg l^{-1} kinetin, plantlets were obtained with less than 1% of albinos. "Explant source had no affect on frequency or quality of plantlets." Ma *et al.* (2004) induced calli from mature caryopses on MS medium supplemented with 4.5 mg l^{-1} 2,4-D and 0.1 mg l^{-1} BAP, and subcultured them on MS medium containing 4 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BAP, and 5 mg l^{-1} ascorbic acid. The MS regeneration medium included 2 mg l^{-1} BAP, 1 mg l^{-1} NAA, 5 mg l^{-1} $CoCl_2$, and 0.5 mg l^{-1} TDZ. The regeneration frequency was over 90%. The authors thought that $CoCl_2$, an ethylene inhibitor, helped in callus regeneration.

2.2 Development of Transgenic Turfgrass Plants

As in most monocot transformation cases, there are three major approaches to transform turfgrasses: protoplast (by polyethylene glycol (PEG) treatment or electroporation), particle bombardment (also known as microprojectile bombardment or the biolistic method, and can be performed with a Du Pont PDS-1000/He device or a particle inflow gun), or *Agrobacterium tumefaciens* infection. The most frequently used selection systems include *Escherichia coli* hygromycin B phosphotransferase gene (*hph* or *hpt*) and hygromycin B (hyg B) (Gritz and Davies, 1983), or *Streptomyces hygroscopicus* phosphinothricin acetyltransferase gene (*bar*) and bialaphos (active ingredient of herbicide Herbiace®) or phosphinothricin (PPT or glufosinate, active ingredient of herbicides Basta™, Liberty®, Finale®, and Challenge®) (Thompson *et al.*, 1987). The selectable marker genes are usually under control of cauliflower mosaic virus (CaMV) 35S promoter, rice *Act1* promoter, or maize *ubi1* promoter. Quite often, the *E. coli* β-glucuronidase gene (*uidA* or *GUS*) (Jefferson *et al.*, 1987) or the jellyfish green fluorescent protein gene (*gfp*) (Chalfie *et al.*, 1994) is used as a reporter gene to help evaluate the transformation efforts. Polymerase chain reaction (PCR) and reporter gene expression are usually employed to preliminarily screen for putative transgenic plants. However, the genome integration of the transgene in the selection agent-resistant plants has to be confirmed by Southern blot analysis. The Southern hybridization patterns often help distinguish independent transformation events. Expression of the transgene should be demonstrated by

Northern and/or Western analysis, and/or the activity of the enzyme encoded by the transgene.

Wang *et al.* (1992) first reported tall fescue transformation by introducing DNA vectors through PEG treatment into protoplasts isolated from embryogenic suspensions. After transformation, the protoplasts were cultured with agarose bead-type method with nurse cells, and were later selected with either hyg B (200 mg l^{-1}) or PPT (100 or 200 mg l^{-1}). The transformation frequency ranged from 10^{-6} and 10^{-5}. A total of 38 independent, morphologically normal transgenic plants were obtained. Plants with *bar* transgene were resistant to herbicide Basta™. In another report, Ha *et al.* (1992) electroporated protoplasts and recovered regenerated transgenic plants with hyg B selection. They also demonstrated *GUS* reporter gene expression in the transgenic plants. The same group later (Penmetsa and Ha, 1994) studied and optimized the conditions (including DNA concentration, protoplast density, age of the suspension, and field strength used for electroporation) for electroporation transformation of tall fescue protoplasts using *GUS* transient expression assays. Kuai and Morris (1995) found that the age of the suspension, the stage of the growth cycle, the time length of cell wall digestion, and the osmolarity of the transformation medium all affected transient and stable *GUS* reporter gene expression in PEG-mediated protoplast transformation. Dalton *et al.* (1995) tested different hyg B selection schemes in PEG-mediated protoplast transformation and observed that continuous selection at a low concentration (50 mg l^{-1}) yielded the highest transformation frequency, and continuous selection at high level biased selection of transgenic plants with multiple transgene copies. The same group (Bettany *et al.*, 2002) reported the highest co-expression frequency (38%) of two co-transformed vectors when the molar gene ratio was 4:1. Spangenberg *et al.* (1995a) obtained transgenic plants by particle bombardment of embryogenic suspensions and hyg B selection. They observed a twofold improved efficiency in recovering resistance calli when the initial selection (hyg B, a step-up selection scheme from 30 up to 150 mg l^{-1}) was performed in liquid culture medium, compared to a continuous solid medium selection (150 mg l^{-1}). About 35% of the hyg B-resistant calli regenerated into plants. Kuai *et al.* (1999) recovered a transgenic plant line of the *bar* gene by protoplast transformation and PPT selection (1.5–3 mg l), which showed resistance to PPT spray and was fertile. However, with limited number of seeds set by crossing with nontransgenic plants, they did not observe transmission of the *bar* gene to the T_1 progenies through pollen. Cho *et al.* (2000) adjusted concentrations of 2,4-D, BAP, and $CuSO_4$ in the culture medium and obtained regenerative tissues containing "multiple, light green, shoot-meristem-like structures" from mature caryopses (cv. Kentucky 31) cultured under dim-light conditions. They co-transformed the tissue with *hpt*, *bar*, and *GUS* transgenes by particle bombardment, and recovered 8 independent transgenic plant lines from hyg B selection (30–100 mg l^{-1}). Co-expression of two transgenes (*hpt* and *bar* or *GUS*) were 50–75%, and 25% transgenic plants had expression of all the three genes. Bai and Qu (2001b) reported transformation of two elite turf-type cultivars (Coronado and Virtue) by bombardment of embryogenic suspensions and hyg B (250 mg l^{-1}) or bialaphos (3–5 mg l^{-1}) selection. Dalton *et al.* (1998) employed silicon carbide "whiskers" method to transform suspensions and obtained 8 transgenic plants. Bettany *et al.* (2003) first reported *Agrobacterium* transformation of tall fescue. They infected suspensions with *Agrobacterium* strain LBA4404 harboring a "super-binary" vector pTOK233 and recovered two independent transgenic plant lines. Later on, more efficient *Agrobacterium*-mediated transformation was reported by three laboratories using 5–8 weeks of embryogenic calli induced from mature caryopses. Lee *et al.* (2004) found that a combination of strain EHA101 (pIG121Hm), 100 μM acetosyringone, and a 30-min vacuum treatment during co-cultivation yielded the best results: over 20% transformation frequency was achieved when hyg B selection was performed at 50 mg l^{-1} level (cv. Kentucky 31). Wang and Ge (2005a) broke embryogenic calli into small pieces (cv. Jesup and Kentucky 31) and infected them with *Agrobacterium* strains LBA4404 or EHA105 harboring pTOK233 or a pCAMBIA binary vector, and selected the transgenics at 250 mg l^{-1} hyg B. An average of 5.5% overall transformation efficiency (independent transgenic plant lines over the number of intact callus pieces infected) was achieved. No advantage of pTOK233 over pCAMBIA vectors was observed.

Fertile plants were obtained and Mendelian inheritance of transgenes was observed at T_1 generation by PCR analysis. Dong and Qu (2005) transformed calli of elite turf-type cultivars Matador and Coronado, and infected them with *Agrobacterium* strains LBA4404 or EHA105 containing binary vector pCAMBIA1301 with or without pTOK47, a vector similar to pTOK233. Approximately 34% of the calli infected were resistant to hyg B selection (250 mg l^{-1}), and 8% overall transformation frequency was achieved. They observed that a higher level of 2,4-D (5 mg l^{-1}) during callus culture and co-cultivation helped improve the transformation efficiency while inclusion of pTOK47 did not. In all the three reports, low transgene copy number in transgenic plants was observed. Recently, Cao *et al.* (2006) obtained highly regenerative callus lines from mature caryopses culture (cv. Triple A) on a modified N6 medium and found higher pH (6.2) enhanced both calli induction and plant regeneration. After co-cultivation with strain EHA105 (pCAMBIA1301), the calli were cultured on a nonselective preregeneration medium containing 400 mg l^{-1} L-cysteine for 7 days followed by regeneration on a nonselective medium. The selection pressure was imposed at the rooting medium (70 mg l^{-1} hyg B), and putative transgenic plants developed roots. In studies of transgene stability and inheritance in transgenic tall fescue plants, Bettany *et al.* (1998) investigated two transgenic plants in their GUS expression after vegetative propagation (tillering). The plants either had multiple *GUS* gene copies or the transgene had deletion or rearrangement. Considerable variations in GUS enzyme activity, among the tillers and from generation to generation, were observed mostly in the first four generations, and GUS expression seemed to be stabilized by the fifth generation. Southern and DNA methylation analysis could not identify obvious difference between the tillers that had various GUS activities. On the other hand, Z.Y. Wang *et al.* (2003a) studied *hph* and *GUS* transgene inheritance in transgenic plants obtained by particle bombardment through reciprocal crosses. Among over 40 T_1 plants from each cross, an 1:1 segregation ratio was observed by PCR analysis, indicating that both of the transgenes were inherited as a genetic locus, and the transgenes were transmitted by both egg cells and pollen grains. Southern analysis showed the same hybridization pattern among T_0, T_1, and T_2 plants, suggesting a stable inheritance of the transgenes through sexual reproduction. To facilitate inheritance study, Z.Y. Wang *et al.* (2003b) developed a protocol for vernalization of transgenic tall fescue plants. In addition, the same group (Z.Y. Wang *et al.*, 2003c) investigated the field performance of transgenic tall fescue plants in comparison with nontransgenic, regenerated plants through tissue culture and nontransgenic, seed-derived plants. Although the T_0 transgenic and the tissue culture plants were inferior to the seed-derived nontransgenic plants in terms of height, tiller number, and seed yield, no major differences were observed among their progenies. In general, transgenic plants performed similarly to the tissue culture plants, and no major effect was observed from transgene introduction.

Kentucky Bluegrass transformation was first reported by Ha *et al.* (2001). The authors used an approach similar to what they did with tall fescue (Cho *et al.*, 2000) to obtain highly regenerative tissues from mature caryopses (cv. Kenblue) and co-bombarded the explants with three transgenes (*hpt*, *GUS*, and *gfp*) and the bombarded tissues were subjected to hyg B selection, first at 100 mg l^{-1} and later at 30 mg l^{-1}. It took quite a few months of subculture before the resistant, light-green tissues were observed. A transformation efficiency of 2.2% was achieved and 70% of the resistant tissues regenerated into plantlets. Co-expression frequency of two transgenes was 30–40%, and for all three genes was 20%. Gao *et al.* (2006) reported an efficient transformation protocol for Kentucky bluegrass using particle bombardment. The authors induced calli from immature embryos (breeding line DP-37-61), of which 90% were embryogenic. They achieved 22% transformation efficiency with the *hpt* transgene and hyg B (100 mg l^{-1}) selection, or 7.5% with the *bar* transgene and bialaphos selection (2 mg l^{-1}). The *bar* gene transgenic plants were resistant to herbicide Basta™. The authors attributed their high transformation efficiency to a combination of the genotype and explant they employed, and the careful selection of the embryogenic callus lines used for the transformation experiments. *Agrobacterium* transformation of Kentucky bluegrass has been reported. Chai *et al.* (2003) found supplement of cupric sulfate enhanced induction

of embryogenic calli from mature caryopses (cv. Md). Embryogenic calli were infected with strain AGL1 (pDM805) and selection was carried out with bialaphos (1.5–3 mg l^{-1}). Four transgenic lines were obtained and the transgenic plants showed resistance to Basta^{TM} spray.

Stable transformation of perennial ryegrass was first achieved with particle bombardment of a nonembryogenic suspension line, and hyg B resistance callus lines were obtained (Van Der Maas *et al.*, 1994). Spangenberg *et al.* (1995b) optimized conditions of bombardment of single-genotype derived embryogenic suspension lines (cv. Citadel) using a particle flow gun. They found that initial liquid selection and a stepwise increase of hyg B selection (from 50–200 mg l^{-1}) had higher transformation efficiency with 26% bombarded plates yielded resistant calli, of which 23% regenerated. Wang *et al.* (1997) performed PEG-mediated protoplast transformation using a selection system containing the *npt*II gene and kanamycin/G418, which is more often used in dicot transformation. The transformed protoplasts were embedded in agarose with no nurse culture and first subjected to selection at 25 mg l^{-1} kanamycin, followed by selection at 25 and 40 mg l^{-1} G418, respectively. A transformation frequency of 5×10^{-6} was achieved, and 20 green plants were obtained. Fertile plants set seeds after crossing and the transgenes were inherited to T1 progenies. Folling *et al.* (1998) found that the nuclease activity released from protoplasts degraded DNA vectors and decreased transformation frequency. Raising the pH of the transformation buffer to 9 and performing transformation at 0 °C reduced the nuclease activity and improved transformation frequency by nearly fivefold. Similar to what they did in tall fescue, Dalton *et al.* (1998) also described perennial ryegrass transformation using silicon carbide, and recovered a transgenic plant. The same group later found that the transformation efficiency of the particle inflow gun was "considerably higher", and used the approach to transform the suspensions of an agronomically more important diploid cultivar (Dalton *et al.*, 1999). They employed liquid culture selection at the first 2–3 weeks at 50 mg l^{-1} hyg B and then solid culture selection at 75 mg l^{-1} of hyg B, and eventually recovered 6 transgenic plants. Altpeter *et al.* (2000) described a selection scheme using *npt*II gene and paromomycin (100 or 200 mg l^{-1}) after particle bombardment of calli induced from mature and immature embryos and immature inflorescences. Dozens of fertile transgenic plants were generated, and the transformation efficiency ranged from 3.7% to 11.4%. Recently two articles described high efficiency transformation of perennial ryegrass by the *Agrobacterium*-mediated approach. Bajaj *et al.* (2006) induced calli from the longitudinally sliced meristematic regions of seedlings. Among 250 lines, 6 highly regenerable callus lines were selected and maintained as plants *in vitro*, and the calli induced from the meristematic regions of these plant tillers were infected with *Agrobacterium* strain EHA101. Transformed calli were selected with hyg B at 94.8 μM (50 mg l^{-1}) for the first 2 weeks and 151.6 μM (80 mg l^{-1}) for the second round. About 7% transformation frequency was achieved and more than 1000 transgenic plants were obtained. Using a similar *Agrobacterium*-mediated transformation and selection scheme as they did in tall fescue, Cao *et al.* (2006) achieved 23.3% transformation efficiency for perennial ryegrass.

Creeping bentgrass transformation was first achieved by particle bombardment of embryogenic calli and the transgenic plants were identified by the GUS transgene expression without any selection (Zhong *et al.*, 1993). Hartman *et al.* (1994) introduced the *bar* gene into two cultivars of creeping bentgrass through bombardment of embryogenic suspensions, and recovered 55 transgenic plants resistant to herbicide Herbiace®, suggesting they could be used in the golf greens to help solve the *Poa annua* weed problems. Lee *et al.* (1996) introduced the *bar* gene into protoplasts using either PEG treatment or electroporation, and obtained 153 transgenic plants resistant to Herbiace®. Xiao and Ha (1997) reported efficient recovery of *hph* transgenic plants using 200 mg l^{-1} hyg B selection after bombardment of suspensions. Asano *et al.* (1998) found that substitution of $CaCl_2$ with $Ca(NO_3)_2$ and elevated pH (9 to 10) in the buffer improved transient GUS activity in protoplast electroporation transformation, and used the altered buffer to recover *bar* transgenic plants. Dalton *et al.* (1998) obtained 6 transgenic plants from silicon carbide transformation of creeping bentgrass. *Agrobacterium*-mediated transformation of creeping bentgrass was first reported by Yu *et al.* (2000) in which the authors introduced a *gfp* gene and recovered the transgenic

plants by detecting the green fluorescence without any selection. Using *Agrobacterium*-mediated transformation and *bar* gene selection, Luo *et al.* (2004b) obtained a large number of transgenic plants (219 independent transformation events) with stable transformation frequency of 18–45%. The *bar* gene was stably expressed in T_1 plants and a Mendelian inheritance was observed among T_1 populations. About two-thirds of the transgenic plants had a single copy of the *bar* transgene. Han *et al.* (2005) reported *Agrobacterium* transformation of creeping bentgrass and *hyg B* selection. A novel *Agrobacterium*-mediated transformation of creeping bentgrass (cv. Penncross) was reported (Wang and Ge, 2005b) in which the nodes from stolons were cut into half and directly infected by *Agrobacterium*. Green shoots were produced from the infected nodes 4–5 weeks under hyg B selection in a medium containing 4.5 μM kinetin and 0.2 μM 2,4-D. The approach was efficient (6.3–11.3%) and transgenic plants can be quickly recovered. Moreover, this approach bypasses the callus induction and regeneration stages and may save a great deal of efforts in improving tissue culture responses, and has potential to be a way for genetic transformation of recalcitrant grass species. It would be interesting to see whether any transgenic plants obtained this way are chimeric and if not, histologically what kind of cells were transformed and formed the shoots. In other aspects, Fu *et al.* (2006) analyzed the integration sites of the transfer DNA (T-DNA) in transgenic creeping bentgrass and found that, like in other plants, the integration is a rather complicated process and may have diverse mechanisms, such as the microsimilarity-based illegitimate recombinations, and involvement of filler DNA, and/or T-DNA truncation. Fei and Nelson (2004) evaluated four transformation events of roundup-tolerant creeping bentgrass expressing the *cp4 epsps* gene, in fitness-related reproductive traits, such as heading date, anthesis duration, inflorescence length, number of florets per inflorescence, pollen size, and seed set capacity by open pollination. No significant differences were observed between the transgenic plants and the nontransgenic plants obtained through tissue culture.

Agrobacterium-mediated transformation of colonial bentgrass was reported by Chai *et al.* (2004). Embryogenic calli from mature caryopses of cv. Tiger were induced on a MS medium containing 2 mg l^{-1} 2,4-D, and infected with *Agrobacterium* strain LBA4404 (pTOK233) in the presence of 100 μM acetocyringone. The infected calli were selected with hyg B first at 50 mg l^{-1} and later at 70 mg l^{-1}, and the callus regeneration medium contained 50 mg l^{-1} hyg B, and the plantlets were further selected at 20 mg l^{-1} for rooting. After two cycles of selection, 81.2% of the hyg B resistant calli had strong *GUS* expression while roots of all hyg B-resistant plants and some leaves also showed GUS activity. Southern analysis of four randomly chosen hyg B-resistant plants showed integration of the *hpt* transgene in the plant genome.

Spangenberg *et al.* (1994) developed a red fescue protoplast culture system from fresh or cryopreserved suspensions using agarose beads, nurse culture, and regenerated plantlets. They employed the system and introduced the *bar* gene into red fescue by PEG treatment and recovered transgenic plants under selection at high concentration (50 mg l^{-1}) of PPT. The plants survived spray of herbicide Basta™. Using the embryogenic suspensions, the same group (Spangenberg *et al.*, 1995a) later reported red fescue transformation by the biolistic method. Eighty-five percent of the resistant calli regenerated into plantlets. Cho *et al.* (2000) used an approach similar to what they did in tall fescue and Kentucky bluegrass as mentioned above, by generating highly regenerative green tissues and bombardment, and obtained transgenic red fescue plants with a stepwise increased selection of hyg B (30–100 mg l^{-1}). Altpeter and Xu (2000) bombarded embryogenic calli with an *npt*II gene construct and used 100 mg l^{-1} paromomycin for selection. Between 3% and 5% of the bombarded calli of red fescue produced transgenic plants. No transformation of other fine fescues was reported.

Among the warm-season grasses, bermudagrass transformation was first reported in the triploid, interspecific hybrid (*C. dactylon* × *C. transvaalensis*, cv. TifEagle). Zhang *et al.* (2003) induced embryogenic calli from stolons and developed suspensions from them. The suspensions were bombarded with a vector containing the *hpt* gene. Seventy-five transgenic plants were obtained under selection of 200 mg l^{-1} hyg B. The integration and expression of the transgene were stable during vegetative propagation. Goldman *et al.* (2004a)

transformed embryogenic calli of TifEagle with the herbicide-resistant *bar* gene, and recovered 89 transgenic plants from at least 9 transformation events using selection of 5–15 mg l^{-1} PPT. The transgenic plants were resistant to herbicide Liberty®. Flow cytometry revealed that most of the plants (82/89) were hexaploid, which often showed significant variation in leaf length and width from the parent cultivar. However, no detectable difference was observed in amplified fragment length polymorphism (AFLP) analysis between the hexaploid transgenic plants and the triploid parent cultivar plants. Hu *et al.* (2005) reported *Agrobacterium*-mediated transformation of TifEagle with the *hpt* gene and hyg B selection. A total of 24 independent transgenic plant lines were obtained. Since the vector used for transformation also contained the *bar* gene, plants expressing the *bar* gene displayed resistance to herbicide Liberty®. Li and Qu (2004) developed highly regenerable callus lines from tetraploid common bermudagrass (*C. dactylon*) cv. J1224, and recovered transgenic plants from four independent transformation events by particle bombardment and hyg B selection (200 mg l^{-1} at callus level and 100 mg l^{-1} at regeneration stage). Because of the *bar* and *GUS* genes in the vector used for co-transformation, one event had GUS activity and three events were resistant to Liberty®. The group later reported *Agrobacterium* transformation of the same cultivar using both callus lines and suspension cells, and hyg B selection (Li *et al.*, 2005). Flow cytometry analysis indicated that the ploidy level of the transgenic plants was not altered. Attempts to use *bar* gene selection resulted in transgenic calli but no plants were recovered. Similar to the approach used in creeping bentgrass, successful transformation through direct node infection by *Agrobacterium* was reported in triploid, hybrid bermudagrass (cv. TifEagle, Wang and Ge, 2005b).

Japanese lawngrass transformation was first reported using direct gene transfer to protoplasts mediated by PEG (Inokuma *et al.*, 1998). The authors found that 400 mg l^{-1} hyg B was a proper level for the selection of resistant cell colonies. The selection seemed to be quite efficient and about 400 transgenic plantlets were recovered without selection at the regeneration stage. Toyama *et al.* (2003) first reported *Agrobacterium* transformation of the Japanese lawngrass. They observed that removal of 2,4-D and $CaCl_2$ from the infection medium, and extension of the co-cultivation period to 9 days enhanced transient GUS reporter gene expression, and thus used these conditions to perform transformation. Four bialaphos-resistant plants were regenerated per 700 mg calli after a step-up selection at callus and shoot levels (0.5–5 mg l^{-1}) and 10 mg l^{-1} bialaphos at rooting medium. Ge *et al.* (2006) again, successfully applied their "node-infection-by-*Agrobacterium*" approach to Japanese lawngrass and obtained shoots resistant to hyg B selection (75 mg l^{-1}) in 4–5 weeks. Approximately 50% of the resistant plants were transgenic, and a transformation frequency of 6.8% was achieved.

Genetic transformation of buffalograss has been reported (Fei *et al.*, 2005). Embryogenic calli induced from female immature inflorescences of cv. 91–118 were subcultured for 15 months to obtain soft, friable, and highly regenerable calli, which were bombarded with gold particles of 0.75 mm in diameter. A delay of selection (7 or 28 days) yielded about 2% of calli resistant to glyphosate using a vector containing two *cp4 epsps* gene constructs. In total, 77 pieces (2.2%) of calli were resistant to glyphosate (1 or 2 mM) selection and transferred to regeneration medium containing 0.1 mM glyphosate. Three green shoots developed roots in the selection-rooting medium (0.05 mM glyphosate). Trait RUR Lateral Flow Test strips revealed the resistant plantlets had CP4 EPSPS enzyme activity.

Genetic transformation of centipedegrass was recently achieved (Hanna, personal communication). No transformation of St. Augustinegrass or seashore paspalum was reported. However, Lee and Berg (1999) had a patent (US patent 5 948 956) on St. Augustinegrass transformation using bombardment of node segments and direct selection of the regenerated plantlets.

2.3 Useful Genes Used in Turfgrass Transformation and Their Effects

Appreciable progress in trait modifications using transgenic technologies in a variety of turfgrass species demonstrates the great potential of genetic engineering in plant breeding, and suggests ways for genetic improvement of turfgrasses at a much accelerated pace than before (Lee, 1996;

Chai and Sticklen, 1998; Wang *et al.*, 2001; Wang and Ge, 2006). Turf varieties with desired traits such as disease, insect, and herbicide resistance and environmental stress tolerance can be expected in the very near future. The use of genetically modified turf varieties on a large scale could benefit the turfgrass industry, lawn owners, and the environment. For example, new genes can be introduced into turf that confer traits such as drought and stress tolerance that will reduce water usage, pest resistance that will reduce pesticide applications, phyto-remediation capability and aluminum tolerance that will improve environmental qualities. So far, a large number of useful genes have been introduced into various turf species to produce transgenic plants with enhanced agronomic traits.

2.3.1 Genes conferring tolerance to biotic stress in turfgrass

Most plants, during their life cycle, frequently encounter biotic and abiotic environmental stresses that adversely affect their growth, development, or productivity. Biotic stresses are imposed to plants either by infection with bacteria, fungi, viruses, insects, and nematodes, or by competition with weeds or other undesirable plant species. Resistance to biotic stress is one of the most important targets in the improvement of turfgrass. Although knowledge of the molecular mechanism involved in biotic stress tolerance of turfgrass is limited, and studies in this area lag far behind other major crop species, information from model species, such as *Arabidopsis* and rice whose genomes have been recently sequenced, and other grass species, provides insights into the complexities of the processes as well as candidate genes to be used in genetic engineering of turfgrass plants with durable resistance to biotic stress.

2.3.1.1 Fungal resistance

Turfgrass species are highly susceptible to a wide range of destructive fungal pathogens and require extensive application of fungicides to maintain turf quality. This not only adds to operational costs, but also raises environmental concerns. Plants have evolved mechanisms to protect themselves from fungal attacks, including coordinated activation and expression of a number of genes encoding pathogenesis-related (PR) proteins, or the expression of specific resistance (R) genes for certain pathogens. In addition, genes identified from sources other than plant kingdom also have been demonstrated to confer disease resistance in plants. With the rapid development of transgenic techniques for use in different turfgrass species, a great number of different disease resistance mechanisms have been genetically engineered into turfgrass species to strengthen the host resistance to fungal attacks and demonstrated to be quite effective, pointing to the great potential of exploiting more in this path for use in practice.

Chitinase and glucanase Chitinase, identified in a wide variety of plants, is one of the most representative pathogenesis response-related proteins (PR3) that are induced strongly during fungal infection, treatment of plant tissues with fungal cell wall extracts, or wounding (Collinge *et al.*, 1993; Graham and Sticklen, 1994). Plant chitinases can attack pathogens directly by degrading chitin (a fungal cell wall component) to confer disease resistance, or by binding to the chitin of the fungal cell wall to interfere with fungal growth because of the affinity of chitin-binding proteins to nascent chitin, which leads to severe morphological changes in fungi (Nielsen *et al.*, 1997; Theis and Stahl, 2004). Chitinases are monomers of 25–35 kDa, found as acidic or basic forms, and consist of a putative chitin binding domain as well as a catalytic domain (Shinshi *et al.*, 1990). β-1,3-glucan is a major component of fungal cell walls. Plants have evolved to have β-1,3-glucanases (PR2) as one of the major PR proteins in their defense systems (De Lucca *et al.*, 2005). β-1,3-glucanases are believed to be able to directly digest the β-1,3-glucans of fungal cell walls, resulting in cell lysis, and/or release of bioactive cell wall fragments as elicitors for inducing plant defense reactions (Leubner-Metzger and Meins, 1999). Both chitinase and β-1,3-glucanase have been introduced into turfgrass aiming at engineering enhanced fungal resistance. Chai *et al.* (2002) transferred the elm chitinase gene (*hs2*) to creeping bentgrass, a cool-season grass mainly used in golf greens.

Creeping bentgrass is susceptible to several fungal diseases, especially *Rhizoctonia solani* (Kuhn), a basidiomycete fungus and the causal agent of brown patch disease, and *Sclerotinia homoeocarpa*, which causes dollar spot disease. Compared to the untransformed plants, two transgenic lines out of five showed improved resistance to brown patch disease. Interestingly, the same group also demonstrated the prevention of fungal diseases in transgenic creeping bentgrass expressing herbicide-resistant gene, *bar*, after spraying of transgenic plants with bialaphos and glufosinate (Liu *et al.*, 1998). This observation was confirmed in separate studies when both a rice chitinase *RCH 10* gene and an alfalfa glucanase *AGLU1* gene were co-transferred with a *bar* gene into creeping bentgrass. Although the transgenic plants themselves did not exhibit resistance to these two fungal pathogens, they became resistant to both dollar spot and brown patch diseases when the herbicide glufosinate was sprayed before the fungal inoculation (Y. Wang *et al.*, 2003a, b). Chitinase also has been introduced into Italian ryegrass (*Lolium multiflorum* Lam.), one of the most important temperate pasture grasses, and sown for forage and turf, to improve plant resistance to crown rust (*Puccinia coronata*), the most serious foliar fungal disease (Takahashi *et al.*, 2005). An *RCC2* gene encoding rice chitinase (*Cht-2*) (Nishizawa *et al.*, 1993) was used to transform Italian ryegrass. Bioassay of detached leaves indicated increased resistance to crown rust in transgenic plants, which exhibited higher chitinase activity than a nontransgenic plant (Takahashi *et al.*, 2005). A recent study in introducing the alfalfa glucanase *AGLU1* gene into tall fescue (*F. arundinacea* Schreb.), a cool-season turf and forage grass species of great economic importance, has resulted in transgenic plants with enhanced resistance to various fungal diseases (Dong *et al.*, 2007). Among the 12 tall fescue plants containing the *AGLU1* gene, two were resistant to brown patch (*R. solani*), and three were highly resistant to another fungal disease, gray leaf spot, caused by an ascomycete fungus of *Magnaporthe grisea* (T.T. Hebert) Yaegashi and Udagawa.

Thaumatin-like proteins (TLPs) Group PR5 of the PR proteins are known as thaumatin-like proteins (TLPs) because of their homology to the sweet-tasting thaumatin from *Thaumatococcus daniellii* (Edens *et al.*, 1982). PR5 proteins have been found to have antifungal activity, presumably by disrupting fungal plasma membrane (Vigers *et al.*, 1992). A rice thaumatin-like protein gene, *tlpd34*, under the control of maize ubiquitin (*ubi1*) promoter was introduced into creeping bentgrass and the transgenic plants expressing TLPD34 showed improved resistance to dollar spot in the field tests although the same transgenic lines showed no improvement against brown patch under greenhouse conditions (Fu *et al.*, 2005). In a separate study, an *Arabidopsis thaliana* (L.) Henyh gene encoding for PR5K, a receptor protein kinase that has an extracellular domain with similarity to the PR5 proteins, was also placed under the control of maize *ubi1* promoter, and introduced into creeping bentgrass. Four of the eight transgenic lines expressing PR5K exhibited delays in development of dollar spot disease symptoms by 29–45 days, relative to the control plants after inoculation (Guo *et al.*, 2003).

Plant resistance (R) genes Plant R genes express in response to the invasion of certain pathogens. They consist of common motifs such as a nucleotide binding site (NBS), leucine-rich repeats (LRRs), kinase, coiled-coil domain (CC), Toll/interleukin-1-receptor (TIR), and transmembrane domain (Hammond-Kosack and Jones, 1997). The R gene products recognize corresponding avirulence gene products (elicitor) from the pathogen, which trigger the cascade of plant defense responses, such as hypersensitive response or programmed cell death, and consequently slow down or completely stop the growth of the pathogen (Dangl *et al.*, 1996; Pennell and Lamb, 1997). *Pi9*, an unusual rice blast R gene that exhibited high resistance to all the rice blast-causing races of *M. grisea* tested so far (Qu *et al.*, 2006), was recently introduced into tall fescue as a genomic DNA fragment with its own gene expression regulatory element and transcriptional terminator. Preliminary results obtained from the transformed plant showed that *Pi9* conferred a high level of resistance to the turfgrass isolates of *Magnaporthe grisea* (Dong *et al.*, 2007).

Ribosome-inactivating proteins (RIPs) RIPs are toxic N-glycosidases that cleave a highly conserved sequence of the 28S rRNA (Endo and Wool, 1982; Theis and Stahl, 2004). RIPs identified in many plant species are believed to function as defense proteins (Stirpe *et al.*, 1992; Barbieri *et al.*, 1993; Nielsen and Boston, 2001). Three types of RIPs from pokeweed (*Phytolacca americana*), referred to as pokeweed antiviral proteins (PAPs), PAP-Y, PAP-C, and PAPII (Irvin *et al.*, 1980; Irvin, 1983) were used to transform creeping bentgrass under the control of maize *ubi1* promoter. Transgenic plants produced exhibited different levels of PAP expression, some of which displayed resistance to dollar spot disease (*S. homoeocarpa*) albeit toxic effect of highly expressed PAPs on host plants (Dai *et al.*, 2003).

Lysozymes Lysozymes exist widely in microorganisms and animals, and are generally considered to be antibacterial because of their 1,4-β-N-acetylmuramidase activity against peptidoglycan in bacterial cells (Jolles and Jolles, 1984). Some lysozymes can hydrolyze chitin and thus also have antifungal activities. Antifungal activity of T4 lysozyme was first observed after abolishing the muramidase activity by heat treatment, and the C-terminal of the enzyme was considered to confer this "membrane-disturbing" activity (Düring *et al.*, 1999) confer resistance to fungal attack in transgenic rice, where over 80% of the transgenic plants expressing T4 lysozyme were resistant to four isolates of the rice blast pathogen *M. grisea* (Tian *et al.*, 2002). This demonstrates the great potential of T4 lysozyme for use in plants in defending fungal attacks. T4LYS, the T4 phage lysozyme gene driven by maize *Ubi1* promoter was introduced into tall fescue to test its utility in conferring disease resistance to transgenic plants (Dong *et al.*, 2008). Among the 14 transgenic plants obtained, seven showed highly significant resistance to gray leaf spot (*M. grisea*). Moreover, three of these plants were also resistant to brown patch disease (*R. solani*). The results demonstrated the potential to introduce T4 lysozyme gene into plants for fungal disease resistance.

Small antimicrobial peptides Fungi, insects, animals, and humans all possess genes encoding antimicrobial proteins or peptides. Many antimicrobial proteins/peptides have been identified and some have been introduced into plants for enhanced disease resistance. Small antimicrobial peptides also can be engineered into plants for disease resistance. Typically, these antimicrobial peptides permeabilize the cell membrane or cause osmotic shock to the pathogens (Nissen-Meyer and Nes, 1997). Dermaseptins are small antimicrobial peptides originally isolated from frog skin. Dermaseptins and their truncated analogs were cytolytic to bacteria, yeast, filamentous fungi, and protozoa (Mor and Nicolas, 1994). Immunofluorescence, electron microscopic, and electrophysiological studies indicated that the interactions between the peptide and the lipid bilayer of cells caused changes in membrane functions, which resulted in the imbalance of the osmotic pressure and cell death (Pouny *et al.*, 1992). A derivative of a dermaseptin B gene was reported to confer resistance to fungal pathogens from *Alternaria*, *Cercospora*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Verticillium* genera in transgenic potato (Osusky *et al.*, 2005). Recently, a trucated dermaseptin SI gene encoding a peptide of 18 AA residues (ALWKTMLKKLGTMALHAG), which was demonstrated to exhibit active antimicrobial activity (Mor and Nicolas, 1994), was introduced into tall fescue under the control of rice *rubi3* gene promoter (Dong *et al.*, 2007). The two transgenic plants expressing the truncated dermaseptin SI gene grew normally and displayed good resistance to gray leaf spot (*M. grisea*). One of them tested was also resistant to brown patch disease (*R. solani*). This preliminary result, together with the results reported in potato (Osusky *et al.*, 2005), suggests the potential of using dermaseptins to render resistance to a wide spectrum of fungal pathogens in turfgrass.

2.3.1.2 Virus resistance

Virus disease is another biotic stress that has big impact on certain turfgrass species. Plant-derived virus resistance genes are often unavailable. Pathogen-derived resistance (PDR) (Sanford and Johnston, 1985) mediated by transgene (virus coat protein genes, replicase genes, or movement protein genes) products, either the proteins (protein-mediated) or the transcripts

(RNA-mediated), have been demonstrated to be very effective in fighting against viruses in plants (Baulcombe, 2005). RNA-mediated virus resistance may operate by targeted degradation of the transgene and homologous virus RNA, resulting in post-transcriptional gene silencing (PTGS) along with inhibition of virus RNA replication. This strategy was used in perennial ryegrass (*Lolium perenne* L.), the most important grass sown in areas with a temperate climate, to genetically engineer resistance to a common perennial ryegrass pathogen, ryegrass mosaic virus (RgMV), which can tremendously reduce plant yield and persistence (Xu *et al.*, 2001). RgMV belongs to the family Potyvirideae, genus *Rymovirus*, and is a cytoplasmically replicating virus with a monocistronic ssRNA genome. An untranslatable RgMV coat protein gene (*RgMV-CP*) driven by the rice *Act1* promoter was introduced into perennial ryegrass. Primary transgenic plants and their sexual progeny exhibited resistance against high-dose virion inocula of different RgMC strains over a 9-month monitoring period after inoculation.

2.3.2 Genes conferring tolerance to abiotic stress in turfgrass

Abiotic stresses arise from an unfavorable physical or chemical environment surrounding the plant, such as water deficit or flooding, high or low temperatures, excessive soil salinity, inadequate mineral nutrients, or presence of pollutants, such as heavy metals in the soil and chemicals, such as herbicides. Many factors interact to determine how plants respond to environmental stresses, including the features of the plants (genotype, developmental stages, and organ or tissue identity), the way a stress is imposed (duration, severity, and frequency) and the additive or synergistic effect of multiple stresses (Bray *et al.*, 2000). Although none of the mechanisms by which higher plants perceive abiotic stresses have been elucidated, tremendous progress in understanding plant responses to stress through different mechanisms has been made (Bohnert *et al.*, 1995; Shinozaki and Yamaguchi-Shinozaki, 1999; Bray *et al.*, 2000; Shinozaki *et al.*, 2003). This knowledge allows designing molecular strategies to genetically modify plants of various species, including turfgrass, for enhanced abiotic tolerance, leading to improved agricultural production.

2.3.2.1 Drought and salt tolerance

Drought and salinity are the most important osmotic stresses affecting plant growth and productivity. Plants respond to water deficit and adapt to drought and salinity conditions by changes in metabolism and development that can often be attributed to altered patterns of gene expression. Many higher plants have evolved mechanisms such as the accumulation of osmoprotectants including amino acids, ammonium compounds, and polyols/sugars to protect themselves from drought and salt stress conditions. This provides tolerance to the cells under stress by stabilizing the quaternary structure of the complex proteins and adjusting the osmotic potential in their cytoplasm to maintain water content. Recent progress has been made in analyzing the complex cascades of gene expression in drought and cold stress responses, especially in identifying specificity and cross talk in stress signaling (Shinozaki *et al.*, 2003; Siobhan *et al.*, 2003).

Several of the stress-induced genes are regulated by ABA, a plant hormone that is increased through *de novo* synthesis in response to water deficit and low temperature. ABA plays a role in several responses to water stress, most notably stomatal closure and induction of gene expression (Crozier *et al.*, 2000; Trewavas, 2000). This suggests the great potential of manipulating ABA levels in plants by enhanced expression of key regulatory genes in ABA biosynthesis for mitigating plant tolerance to water stress, and has been demonstrated in transgenic *Arabidopsis* (Iuchi *et al.*, 2001) and tobacco (Qin and Zeevaart, 2002) plants overexpressing a gene for 9-cis-epoxycarotenoid dioxygenase (Qin and Zeevaart, 1999) that catalyzes the cleavage of 9-cis-epoxycarotenoids, the first committed and presumably the limiting step of ABA biosynthesis. Recently, *VuNCED1*, a *9-cis-epoxycarotenoid dioxygenase* gene cloned from cowpea (Iuchi *et al.*, 2000) and placed under the control of the CaMV 35S promoter was introduced into creeping bentgrass (Aswath *et al.*, 2005). Challenge studies performed with transgenic plants by exposure to water stress (up to 75%) and salt stress (up to 10 $dS\,m^{-1}$) for

10 weeks, revealed that more than 50% of the transgenic plants could survive drought and NaCl stress whereas wild type was not. ABA levels were measured under drought and normal conditions; endogenous ABA was dramatically increased by drought and NaCl stress in transgenic plants.

The detrimental effects of salt on plants are a consequence of both a water deficit resulting in osmotic stress and the effects of excess sodium ions on key biochemical processes. To tolerate high levels of salts, plants should be able to use ions for osmotic adjustment and to internally distribute these ions to keep sodium away from the cytosol. Vacuolar Na^+/H^+ antiports, the prevalent membrane proteins, found in animals, yeasts, bacteria, and plants (Fukuda *et al.*, 1999), play important roles in this process. They function to pump Na^+ from cytoplasm into vacuole, to maintain a higher K^+/Na^+ ratio in the cytoplasm than that in vacuoles, protecting cell from sodium toxicity (Fukuda *et al.*, 1999). In addition, the vacuolar proton pumps, such as the vacuolar H^+-pyrophosphatase, also should increase the sequestration of ions in the vacuole by increasing the availability of protons. Although salt tolerance is a complex trait that seems to involve a large number of salt-responsive genes (Zhu, 2000), the overexpression of a single gene, such as the vacuolar Na^+/H^+ antiport gene (Apse *et al.*, 1999; Zhang and Blumwald, 2001; Zhang *et al.*, 2001; Ohta *et al.*, 2002; Fukuda *et al.*, 2004) or the H^+-pyrophosphatase gene (Gaxiola *et al.*, 2001; Park *et al.*, 2005), has been shown to improve plant tolerance to salt, and in some cases, to drought in transgenic *Arabidopsis*, tomato, and rice plants. A rice Na^+/H^+ antiport gene, *OsNHX1* driven by the CaMV 35S promoter has been introduced into perennial ryegrass (*L. perenne* L.) (Wu *et al.*, 2005). The resultant transgenic ryegrass had better salt tolerance. After stress treatment for 10 weeks with 350 mmol l^{-1} NaCl, transgenic plants survived, while wild-type plants did not. The leaves of transgenic plants accumulated higher concentrations of Na^+, K^+, and proline than those of the control plants.

2.3.2.2 Freezing and cold tolerance

Fructans are polyfructose molecules produced from the polymerization of sucrose that accumulate in plants in addition to or instead of starch (Hendry, 1993). Fructans are particularly widespread in the grasses and predominantly stored as highly accessible, nonstructural carbohydrates in the vacuole where they are synthesized (Chatterton *et al.*, 1989; Hendry, 1993). Fructan not only plays an important role in assimilate partitioning (Pollock and Cairns, 1991; Schnyder, 1993), but also has been associated with improved tolerance to cold and drought through osmoregulation (Pontis, 1989; Pollock and Cairns, 1991; Hendry, 1993; Ebskamp *et al.*, 1994; Pilon-Smits *et al.*, 1995; Konstantinova *et al.*, 2002). The *sacB* gene for *Bacillus subtilis* levansucrase, one of the enzymes involved in the synthesis of high-molecular-weight bacterium fructan (levan) was placed under the control of the maize *ubi1* promoter, or the double CaMV 35S promoter and introduced into Italian ryegrass (*Lolium multiflorum* Lam.) (Ye *et al.*, 2001). In order to direct the expressed levansucrase to the vacuole (Ebskamp *et al.*, 1994), the *sacB* coding sequence was fused to the vacuolar targeting signal sequence from the yeast carboxypeptidase Y (*cpy*) or sweet potato preprosporamin (*spor*) genes (Hattori *et al.*, 1985; Valls *et al.*, 1987). Transgenic plants expressing the chimeric *sacB* gene accumulated low levels of bacterial levan and displayed distorted native grass fructan synthesis pattern and slowed growth with the onset of the reproductive phase. It is unclear how this was related to the response of transgenic plants to environmental stresses. In a separate study, transgenic perennial ryegrass (*Lolium perenne*) plants were produced that overexpressed the CaMV 35S promoter-driven wheat fructosyltransferase genes, *wft1* and *wft2*, which encode sucrose-fructan 6-fructosyltransferase (6-SFT) and sucrose-sucrose 1-fructosyltransferase (1-SST) (Kawakami and Yoshida, 2002), respectively. Significant increases in fructan content and freezing tolerance on a cellular level were detected in the transgenic perennial ryegrass plants (Hisano *et al.*, 2004).

Cytokinins, a class of phytohormones, play an important role in the processes of plant development. They have pleiotropic effects on plants when applied exogenously, including shoot initiation from callus cultures, promotion of axillary bud growth, directed transport of nutrients, stimulation of pigment synthesis, inhibition of root growth, and delay of senescence (Medford

et al., 1989). *ipt*, the *A. tumefaciens* cytokinin biosynthetic gene coding for the enzyme isopentenyl transferase involved in the rate-limiting step in cytokinin biosynthesis (Akiyoshi *et al.*, 1983), and has been transferred into plants to induce additional cytokinin production as well as a number of modifications in morphology and plant development (Li *et al.*, 1992; Hewelt *et al.*, 1994; Faiss *et al.*, 1997; Rupp *et al.*, 1999), some of which indirectly impacted on stress tolerance (Smigocki *et al.*, 1993; Li *et al.*, 2004). The *A. tumefaciens ipt* gene driven by maize *ubi1* promoter was introduced into *F. arundinacea*, a cool season tall fescue turfgrass. Transgenic plants expressing exogenous *ipt* gene showed enhanced tillering ability, higher chlorophyll a and b levels, a longer stay-green period under low-temperature conditions and consequently, greatly improved cold tolerance (Hu *et al.*, 2005).

2.3.2.3 Transcription factors for use in abiotic stress tolerance

Abiotic stress-inducible transcription factors are capable of activating the expression of multiple downstream genes involved in protection against environmental stresses, thus leading to a wide-arrayed altered response. The transcription factor of the C-repeat/dehydration-responsive-element (C/DRE) pathway, DREB1A/CBF3 specifically interacts with the dehydration responsive element (DRE/CRT) of promoters of the responsive genes. Overexpression of *DREB1A* in transgenic plants of various species enhanced tolerance to different abiotic stresses with concomitant expression of target genes (Kasuga *et al.*, 1999, 2004; Jaglo *et al.*, 2001; Hsieh *et al.*, 2002a, b; Oh *et al.*, 2005). These results demonstrate the great potential of transgenic expression of regulatory genes as a more effective approach for enhancing plant stress tolerance. A DREB1A transcription factor ortholog BCBF3 isolated from wild barley and under the control of the stress-inducible barley *HVA1* promoter was introduced into bahiagrass (*Paspalum notatum* Flugge), an important warm season perennial grass to evaluate the effects of stress-inducible transgene expression on the response of transgenic plants to abiotic stress, and demonstrated to confer enhanced freezing and drought tolerance (James *et al.*, 2004).

2.3.3 Genes conferring tolerance to herbicides in turfgrasses

Weeds or other undesirable plant species (including turf species themselves) greatly impact turf quality for a given turfgrass species and may be considered to represent another type of biotic stresses. The use of herbicides to reduce loss in agricultural production due to weeds has become an integrate part of modern agriculture. The application of herbicides for controlling weeds in turfgrass calls for turf varieties with tolerance to different herbicides. Herbicide resistance in turfgrass through genetic engineering provides a very effective tool in golf course management and lawn maintenance.

2.3.3.1 Resistance to PPT, bialaphos, or glufosinate herbicide

Bialaphos herbicide is a tripeptide antibiotic produced by *S. hygroscopicus*, an organism that produces the tripeptide bialaphos as a secondary metabolite. It consists of two L-alanine residues and PPT, an analog of L-glutamic acid, which is an inhibitor of glutamine synthetase (GS). GS plays a central role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants. It is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation, and photorespiration. Inhibition of GS by PPT causes rapid accumulation of ammonia, which leads to death of the plant cell (De Block *et al.*, 1987). While PPT is an inhibitor of glutamine synthetase in both plants and bacteria, the intact tripeptide has little or no inhibitory activity *in vitro*. In both bacteria and plants, intracellular peptidases remove the alanine residues and release active PPT. The *bar* gene originally cloned from *S. hygroscopicus* is involved in the bialaphos biosynthesis pathway. It encodes a modifying enzyme, phosphinothricin acetyltransferase (PAT) that acetylates the free NH_2 group of PPT and thereby prevents autotoxicity in the producing organism (Thompson *et al.*, 1987). Since its first introduction into plants as a selectable marker in plant transgenic research (De Block *et al.*, 1987), it has been widely used in various plant species, including a number of turfgrass species (Wang

et al., 1992; Hartman *et al.*, 1994; Spangenberg *et al.*, 1994; Lee, 1996; Asano *et al.*, 1998; Liu *et al.*, 1998; Yu *et al.*, 2000; Y. Wang *et al.*, 2003a, b; Luo *et al.*, 2004b). A commercial creeping bentgrass cultivar that has been genetically engineered for glufosinate resistance and male sterility is currently under field trial (Luo *et al.*, 2004a, b, 2005).

2.3.3.2 Resistance to glyphosate herbicide

Glyphosate herbicide (marketed under the trade name Roundup) inhibits 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) within the aromatic amino acid biosynthetic pathway. Disruption of this pathway not only creates a deficiency in protein synthetic precursors, but also affects many other plant cell components that are derived from intermediates and derivatives of this pathway (e.g., auxins, lignans, flavonoids, anthocyanins, and quinones). Therefore, a crop plant must be engineered with a resistant enzyme to maintain flux through this pathway for uninhibited growth and development. A naturally occurring form of EPSPS from *Agrobacterium* sp. strain CP4 provides tolerance to high concentrations of glyphosate. Transgenic expression of CP4 EPSPS within glyphosate-tolerant Roundup Ready® soybean, cotton, and canola provided the appropriate support to the aromatic amino acid biosynthetic pathway without negative impact on yield, compositional qualities, and nutritional value of the harvested product. The *Agrobacterium* CP4 EPSPS also has been genetically engineered into creeping bentgrass (*A. stolonifera* syn. *A. palustris*) to create Roundup-resistant transgenic lines that are currently in the process of applying for the United States Department of Agriculture (USDA) deregulation (Gardner *et al.*, 2003, 2004; Fei and Nelson, 2004).

2.3.3.3 Conversion of proherbicide to herbicide for selective elimination of undesirable plants

The *E. coli argE* gene encodes *N*-acetylornithinase in the *E. coli* arginine biosynthetic pathway, which removes the acetyl group from N^2-acetylornithine and produces acetate and ornithine (Meinnel *et al.*, 1992). The enzyme can also de-acetylate N-acetyl-PPT, a nontoxic compound, to produce PPT (Kriete *et al.*, 1996). This property was recently used in turfgrass to develop a strategy to eliminate undesirable plants in golf course management. Perennial ryegrass is widely used for winter overseeding of dormant bermudagrass on golf courses and sports fields in southeastern United States to provide green color and improved playability. Late spring and summer persistence of perennial ryegrass may decrease the quality of the bermudagrass turf and reduce its winter hardiness. To solve this problem, the *E. coli argE* gene under the control of maize *ubi1* promoter was introduced into perennial ryegrass and transgenic plants expressing the *argE* transgene were selectively controlled upon application of the nontoxic proherbicide, N-acetyl-PPT. The nontransgenic bermudagrass plants were unaffected by the treatment (Chen *et al.*, 2005). This approach provides a means to selectively remove a group of transgenic plants without affecting other plants growing with them.

2.3.4 Modification of other important traits in turfgrasses

2.3.4.1 Modification of senescence

Senescence-associated gene promoters, whose functional values have been demonstrated in dicot genetic manipulation (Gan and Amasino, 1997), are of considerable interest in grasses because of their probable involvement in economically important traits such as productivity (Thomas and Howarth, 2000), sward color (Thorogood, 1996), and responses to elevated CO_2 (Ludewig and Sonnewald, 2000). The 5′ flanking sequence of a maize *SEE1* gene encoding cysteine protease was used to drive the *A. tumefaciens* cytokinin biosynthesis gene, *ipt,* in ryegrass (*L. multiflorum* Lam.). Transgenic analysis demonstrated that the *SEE1* flanking sequence functioned as a senescence-enhanced promoter in ryegrass. The *ipt* transgene was detected in 28 regenerants from five independent transformation events, and the leaves of transgenic plants displayed a stay-green phenotype. Some lines developed spontaneous lesions (Li *et al.*, 2004).

2.3.4.2 Floral inhibition

Flowering in plants is controlled by both environmental (primarily temperature and light) and genetic factors. The genetic factors include genes encoding transcriptional activators and repressors, such as MADS box genes, zinc-finger transcription factors, AP2-domain genes, MYB-domain genes, and also RNA-binding proteins, polycomb-group genes, starch metabolism related proteins, photoreceptors, circadian-clock controlling proteins, GA biosynthesizing enzymes, GA signaling repressors, etc. (reviewed in Levy and Dean, 1998; Simpson *et al.*, 1999). The genes that control the transition from vegetative to reproductive growth called floral meristem identity genes, act either by repressing or promoting flowering. For example, *TERMINAL FLOWER1* (*TFL1*), a floral meristem identity gene, identified in *Arabidopsis* has been demonstrated to specify an indeterminate identity of inflorescence meristems and extend plant vegetative phase. *Arabidopsis* plants overexpressing *TFL1* flower late and produce more secondary shoots (Ratcliffe *et al.*, 1998). Similar functions have been demonstrated in its homolog, *LpTFL1* isolated from perennial ryegrass (*L. perenne* L.). Expression of *LpTFL1* in *Arabidopsis* gave a remarkably strong phenotype with a dramatic delay in flowering (some nonflowering) and extended lateral branching (Jensen *et al.*, 2001).

In turfgrass, the stems that start to emerge during the growth season suppress the formation of new shoots and affect the quality, density, and persistence of the sward. Therefore, prolonged vegetative growth phase through delay of flowering is of major interest in genetic improvement of turfgrass. To manipulate the transition to flowering in turfgrass, the strong floral repressor, *LpTFL1* gene was introduced into red fescue (*F. rubra* L.), a commercially important cool-season turfgrass. Expression of *LpTFL1* with the constitutive maize *ubi1* promoter represses flowering in red fescue, and the flowering repression phenotype correlates well with the level of *LpTFL* expression. Transgenic lines showing low to intermediate expression of *LpTFL1* flowered approximately 2 weeks later than the controls, and transgenic lines showing very high *LpTFL1* expression levels still remained nonflowering after exposure to natural vernalization conditions (Danish winter) in two successive years. There were no other phenotypic effects associated with the *LpTFL* transgene expression during vegetative growth (Jensen *et al.*, 2004).

The *Arabidopsis* TALE-homeobox gene *ATH1* is highly expressed in the shoot apical meristem (SAM) and leaf primordia of the seedlings (Quaedvlieg *et al.*, 1995; Bürglin, 1997). Prior to floral transition, *ATH1* SAM expression is gradually down-regulated to undetectable levels. *Arabidopsis* plants constitutively expressing antisense *ATH1* and *ath1* mutants display a flowering time phenotype that suggests that *ATH1* functions as an inhibitor of floral transition (Van Der Valk *et al.*, 2004). In addition, the tobacco plants constitutively expressing *ATH1* exhibit late flowering as a consequence of impaired GA biosynthesis (Van Der Valk *et al.*, 2004). To delay floral transition in turfgrass, the *Arabidopsis ATH1* gene driven by the maize *ubi1* promoter, the rice actin *Act1* promoter, or the rice *OSH1* promoter, respectively, was introduced into perennial ryegrass. In *ATH1*-expressing plants heading was delayed, and in a number of cases the plants never flowered at all. Such non- or late heading was accompanied by the outgrowth of normally quiescent lateral meristems into extra leaves, resulting in a leafy growth habit. When eventually heading, these plants generally produced a reduced number of inflorescences (Van Der Valk *et al.*, 2004).

2.3.4.3 Down-regulation of pollen allergens

Perennial ryegrass (*L. perenne* L.) and Italian ryegrass (*L. multiflorum* Lam.) are major sources of allergenic pollen causing hay fever and seasonal allergic asthma in humans during the flowering period in spring and summer in cool temperate climates. To reduce the allergic potential of ryegrass pollen, the expression of three main allergens of ryegrass pollen, proteins Lol p 1 (35 kDa), Lol p 2 (11 kDa), and Lol p 5 (31 kDa), have been down-regulated in transgenics. The introduction of the *Lol p 1* and *Lol p 2* transgenes in antisense orientation under control of the maize pollen specific *Zm13* promoter into perennial ryegrass and Italian ryegrass led to a reduction in accumulation levels of Lol p 1 and Lol p 2 allergens in pollen of transgenic plants, as

demonstrated by immunoblots using polyclonal antibodies raised against the recombinant Lol p 1 and Lol p 2 allergens as well as with sera from grass pollen-sensitized patients (Petrovska *et al.*, 2004). Similarly, down regulation of the Lol p 5 allergen in annual ryegrass *L. rigidum* L. was achieved with an antisense construct under control of a pollen-specific promoter, *Ory s1*. Immunoblot analysis of proteins with allergen-specific antibodies did not detect Lol p 5 in the transgenic pollen. The transgenic pollen showed remarkably reduced allergenicity as reflected by low IgE binding capacity of pollen extract as compared with that of control pollen. The transgenic ryegrass plants in which *Lol p 5* gene expression is perturbed showed normal fertile pollen development (Bhalla *et al.*, 1999).

2.4 Site-Specific DNA Recombination for Genome Modification in Turfgrass

Site-specific DNA recombination is a precisely defined DNA rearrangement between two appropriate target sequences. A number of site-specific recombinases have been identified in bacteria and yeast that catalyze DNA recombination between specific target DNA sites (Ow and Medberry, 1995), producing various recombinant molecules according to the orientation of their specific target sites. Recombination between directly oriented sites on a circular molecule leads to excision of the DNA between them, whereas recombination between inverted target sites causes inversion of the intervening DNA. Recombination between sites on separate molecules produces a co-integration event. Some site-specific recombination systems have been shown to function efficiently in heterologous cellular environments. For example, FLP/*FRT* from the 2 μm plasmid of *Saccharomyces cerevisiae* (Broach *et al.*, 1982), or Cre/*lox* from *E. coli* phage P1 (Austin *et al.*, 1981) have been demonstrated to catalyze DNA recombination in a large number of plant species, including agriculturally important crops (reviewed in Luo and Kausch, 2002; Hu *et al.*, 2006), providing excellent genetic tools for controlled modification of plant genomes including chromosomal deletions, inversions, transpositions (Ow, 1996; Luo and Kausch, 2002), or site-specific gene targeting (Albert *et al.*, 1995; Vergunst and Hooykaas, 1998; Vergunst *et al.*, 1998; Srivastava and Ow, 2002).

To study the feasibility of using FLP/*FRT*, a site-specific DNA recombination system originally identified in yeast, for controlled genome modification in plants of turfgrass species, suspension cell cultures of creeping bentgrass (*A. stolonifera* L.) and Kentucky bluegrass (*P. pratensis*) were co-transformed with a FLP recombinase expression vector and a recombination-reporter test plasmid containing *β-glucuronidase* (*gusA*) gene, which was separated from the maize *ubi1* promoter by an *FRT*-flanked blocking DNA sequence to prevent its transcription. GUS activity was observed in co-transformed cells when conducting transient assays. PCR and Southern analyses indicated that FLP-mediated excision of the blocking sequence had brought into proximity the upstream promoter and the downstream reporter gene, resulting in GUS expression. Functional evaluation of the FLP/*FRT* system using transgenic creeping bentgrass plants stably expressing FLP recombinase confirmed the observation from the experiment using suspension cell culture (Luo and Kausch, 2002; Hu *et al.*, 2006). These results indicate that FLP/*FRT* system is a useful tool for genetic manipulation of turfgrass, pointing to the great potential of further exploiting the system for genome modification in perennials.

2.5 Molecular Strategies for Transgene Containment in Turfgrass

To realize the full potential of agricultural biotechnology, the ecological, commercial, and public interest concerns about transgene escape to wild and nontransformed plants through the dispersal of viable pollen and/or dissemination in seed must be addressed (Wipff and Fricker, 2001; Adam, 2002; Dale *et al.*, 2002; Daniell, 2002; Watrud *et al.*, 2004). This is especially important when the target plant species are perennials and outcrossing, such as turfgrasses. Field trial (Wipff and Fricker, 2001) and landscape-level studies (Watrud *et al.*, 2004) on pollen-mediated gene flow from genetically modified creeping bentgrass (*A. stolonifera* L.), one of the first wind-pollinated and highly outcrossing transgenic perennial crops being developed for commercial use have presented evidence that documents multiple instances

at numerous locations of long-distance viable pollen movement from multiple source fields of genetically modified creeping bentgrass. A recent study using glyphosate-resistant creeping bentgrass plants expressing the *CP4 EPSPS* transgenes demonstrates that transgene flow from short-term production can result in establishment of transgenic plants at multikilometer distances from genetically modified source fields or plants. Selective pressure from direct application or drift of glyphosate herbicide could enhance introgression of *CP4 EPSPS* transgenes and additional establishment. Obligatory outcrossing and vegetative spread could further contribute to persistence of *CP4 EPSPS* transgenes in wild *Agrostis* populations, both in the presence or absence of herbicide selection (Reichman *et al.*, 2006). These findings suggest that implementation of strategies for gene containment in transgenics might be useful for field release of any transgenic turfgrass species in the near future.

2.5.1 Male sterility for gene containment

Male sterility resulting from the lack of significant numbers of viable pollen grains, when linked to the genes of interest that are to be transferred into the target species, provides an effective way for interrupting gene flow. The feasibility of using cell-specific expression of cytotoxic molecules and an antisense gene that controls male fertility to block pollen development in transgenic turfgrass has been studied (Luo *et al.*, 2004a, 2005). To induce male sterility in turfgrass, a 1.2-kb regulatory fragment (TAP) of the rice *RTS* gene that is exclusively expressed in the anther's tapetum during meiosis (Luo *et al.*, 2006) was fused with two different genes. One is a ribonuclease gene for barnase from *Bacillus amyloliquefaciens* (Hartley, 1988) and the other, the antisense of the rice *RTS* gene essential for male fertility (Luo *et al.*, 2006). Both chimeric genes were linked to the *bar* gene for selection by resistance to the herbicide glufosinate. *Agrobacterium*-mediated transformation of creeping bentgrass (cv. Penn A-4) with both constructs resulted in herbicide resistant transgenic plants that were also 100% pollen-sterile. Mendelian segregation of herbicide resistance and male sterility was observed in T_1 progeny derived from crosses with wild-type plants. Controlled self- and cross-pollination studies showed no gene transfer to nontransgenic plants from male-sterile transgenics. Thus, male sterility can serve as an important tool to mitigate transgene escape in bentgrass. It could also provide a tool in controlling gene flow in other perennial species using transgenic technology for trait improvement.

2.5.2 Controlled total sterility for gene containment

Another molecular strategy, which combines manipulation of genes involved in the transition from vegetative to reproductive growth and the use of site-specific DNA recombination systems for controlled total vegetative growth has been proposed for mitigating transgene escape in perennials (Oliver *et al.*, 2004; Hu *et al.*, 2006). In this method, a controlled total vegetative growth can be achieved by using down-regulation of a plant gene, the *FLORICAULA/LEAFY* (*FLO/LFY*) homolog, which determines the vegetative to reproductive developmental transition of meristems (Coen *et al.*, 1990; Weigel *et al.*, 1992), together with FLP/*FRT* recombination system for prevention of transgene escape from genetically engineered turfgrass to wild and nontransformed species. Transgenic turfgrass plants containing a construct in which a constitutive promoter is separated from either an RNAi (RNA interference) construction or an antisense of the turfgrass *FLO/LFY* homolog gene by a blocking sequence flanked by directly oriented *FRT* sites can be generated and expected to flower normally to produce seeds. When crossed to a plant expressing transgene of interest together with the FLP recombinase transgene, FLP should excise the *FRT*-flanked blocking fragment thus bringing the RNAi construction or the antisense of turfgrass *FLO/LFY* homolog proximal to the upstream constitutive promoter, activating the constitutive expression of the RNAi or antisense construction for *FLO/LFY*. This in turn will down-regulate the expression of the endogenous *FLO/LFY* genes rendering the plant incapable of producing flowers. The vegetative growth habit of the hybrid retains its commercial application but is incapable of transferring transgenes to neighboring grasses or weedy relatives.

3. REGULATION OF GENETICALLY ENGINEERED TURFGRASSES

As with any genetically engineered (GE) plant in the United States, GE turfgrasses are regulated under the 1986 Coordinated Framework for Regulation of Biotechnology. The US government agencies responsible for oversight of the products of agricultural modern biotechnology are the US Department of Agriculture's Animal and Plant Health Inspection Service-Biotechnology Regulatory Services (USDA-APHIS) (http://www.aphis.usda.gov/biotechnology/brs_main.shtml), the US Environmental Protection Agency (EPA) (http://www.epa.gov/pesticides/biopesticides), and the Department of Health and Human Services' Food and Drug Administration (FDA) (http://www.cfsan.fda.gov).

The Federal laws currently used to regulate the products of modern biotechnology are the Plant Protection Act (PPA), the Federal Food, Drug, and Cosmetic Act (FFDCA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and the Toxic Substances Control Act (TSCA). Regulations have been developed under these statutes as needed to address GE products. New regulations, policy statements, and guidelines continue to be developed as needed.

The responsibilities of USDA, FDA, and EPA in the regulatory oversight of GE crop plants and their products are complementary, and in some cases overlapping. USDA-APHIS has jurisdiction over the planting of GE plants. FDA has jurisdiction over food and feed uses of all foods from plants. EPA has jurisdiction over planting and food and feed uses of pesticides engineered into plants (these are referred to as plant-incorporated protectants, or PIPs). These PIPs would include resistance to diseases and other pests. In addition to PIPs, EPA has jurisdiction over labeling or use of herbicides to be applied on GE herbicide-tolerant plants.

Depending on its characteristics and intended use, a product may be subject to review by one or more of these agencies. A food crop plant being developed using genetic engineering to produce a pesticide in its own tissue provides an example that is reviewed by all three regulatory agencies. A common example of this type of product development is corn into which scientists have inserted a gene isolated from the soil bacterium, *Bacillus thuringiensis* (*Bt*). The *Bt* gene encodes a protein that acts as a pesticide for Leptidoptera and when this gene is inserted into the plant, the plant can then produce the *Bt* pesticidal substance.

USDA-APHIS under the PPA would regulate the experimental corn as a "regulated article". USDA-APHIS oversight begins early in the development cycle and continues until the developer applies for and is granted nonregulated status for the plant provided it can be shown that the plant is not a plant pest. Until such time as the developer is granted nonregulated status for the plant, USDA-APHIS authorizes interstate movement, importation, and field-testing of the plant.

EPA regulates the distribution, sale, use, and testing of the pesticidal substance. In the case of *Bt* in corn, EPA regulates the *Bt* because it is a pesticide. EPA generally controls the field testing of pesticides through an Experimental Use Permit (EUP). In order to legally sell or distribute the pesticide in commerce, the company must register the pesticide with EPA. Through the registration, EPA can establish the conditions of commercial use. EPA is also responsible for setting the amounts or levels of pesticide residue that may safely be in food or feed (i.e., establish a tolerance). EPA may allow an exemption from the requirement to set such a tolerance if it can be shown that there is no food or feed safety issues associated with the pesticide.

Developers of the *Bt* corn also consult with FDA about possible other unintended changes to the food or feed, for example possible changes in nutritional composition or levels of natural toxicants. Although this consultation is voluntary, all of the GE food/feed products commercialized to date have gone through the consultation process. The consultation with FDA serves to ensure that safety or other regulatory issues that fall within the agency's jurisdiction, including appropriate labeling of the food, are resolved prior to commercial distribution.

In the case of GE turfgrasses, these plants are not generally developed for food or feed purposes. If this is the case, FDA would not be involved in the review of the GE plant. However, any of the turfgrasses could be used for forage to be fed to animals. This may happen especially during seed production when the straw is removed from the fields after the seed harvest. In many cases, the

straw is fed to animals. Therefore, if there is a possibility that the GE plants will be used for food or feed, it is highly recommended that the FDA review the product.

Disease resistance and insect resistance have always been important goals in the improvement of turfgrasses. Since these characteristics are considered plant-incorporated pesticides (PIPs), both EPA and APHIS would be involved in regulating these plants. EPA requires EUPs for testing an unregistered PIP or an unregistered use of a PIP on a cumulative total of over 10 acres. USDA-APHIS requires permits for all field testing of regulated GE turfgrass plants regardless of the size of the field trial. APHIS and EPA would both review their respective applications and commercial releases for potential impacts on nontarget organisms such as beneficial insects and threatened and endangered species.

Weed control has always been an important management component for high quality turf and, therefore, incorporation of tolerance to a specific herbicide into a turfgrass could be an important goal for turfgrass variety improvement. In the case of GE herbicide tolerance, USDA-APHIS would be the lead agency in regulating these plants with permits required for all field testing, movements, and importations. Since weeds need to be controlled for optimum turf performance for the herbicide-tolerant grass, the herbicide needs to be applied at least occasionally to the turf. Even if this specific herbicide is registered for many different uses, this new use for its application on this new herbicide-tolerant variety needs to be approved by EPA, and this review and approval would be reflected in the labeled use for this herbicide.

Finally, in the case of GE modifications for other desirable turfgrass characteristics such as less vertical growth, tolerance to various stresses (heat, cold, drought, wear, etc.), nutrient use efficiency, etc., USDA-APHIS would be the only agency regulating field testing, movements, and importations assuming it is not used for feed.

3.1 Regulatory Considerations During Field Testing of Transgenic Turfgrasses

Since USDA-APHIS is the regulatory agency most involved in all phases of field testing of GE plants before commercial release, this discussion will address only USDA-APHIS considerations. APHIS protects agriculture and the environment by ensuring that biotechnology is developed and used in a safe manner. Through a strong regulatory framework, the Biotechnology Regulatory Services (BRS) program of USDA-APHIS ensures the safe and confined introduction of new GE plants with significant safeguards to prevent the accidental release of any GE material. APHIS regulations require that a developer of a GE plant have BRS authorization prior to importing, moving interstate or field testing the GE plant. Applicants must submit all plans for movement, importation, or field testing for thorough review by regulatory scientists. Those scientists will then evaluate the proposed procedures and assess any potential risks. The developer must adhere to certain measures that ensure adequate confinement of the organism. BRS also works closely with states to be sure that they are aware of field tests taking place within their jurisdiction and to allow them to request any additional conditions they may require. To ensure compliance with the permit conditions, BRS inspects field test sites and audits records.

Because each species presents its own unique challenges and opportunities, and regulations (The regulations are in 7 CFR 340 http://www.access.gpo.gov/nara/cfr/waisidx_05/7cfr340_05.html) and guidelines dealing with GE organisms tend to change frequently for this fast changing science, BRS encourages developers of GE turfgrasses to visit the BRS website (http://www.aphis.usda.gov/biotechnology/brs_main.shtml) and with BRS scientists to fully understand the requirements and expectations that need to be met before a GE turfgrass can be field tested and eventually commercialized. Permits for field testing GE turfgrasses may require up to 120 days to process so substantial advanced planning is needed. However, if the permit application involves a new species or a novel modification that raises new issues not previously addressed by APHIS, an environmental assessment may need to be prepared which may require additional time for processing the permit. Previously approved field tests and associated environmental assessments can be viewed online at the website for Information Systems for Biotechnology (http://www.isb.vt.edu). Examining previous environmental assessments will give the developer

a flavor for the types of information considered for an environmental assessment.

For submitting a permit application, the present requirements call for submitting an APHIS Form 2000 (Since regulations and guidelines change frequently, please check the website noted below. Beginning 2007 the permit application may be submitted electronically.) Following are some key features for submitting a permit for field testing a regulated GE plant: the responsible person needs to be a US resident or designate an agent who is a US resident; confidential business information may be claimed (see the BRS website for detailed guidance); scientific and common names of the donor organisms (The organisms from which the DNA was obtained—the typical construct may have pieces of DNA (promoter, gene of interest, terminators, selectable markers, etc.) from several organisms); scientific and common names of the recipient plant; vector or vector agents (biolistics, disarmed *Agrobacterium tumifaciens*, etc.); names or line numbers of the regulated article; a description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the nonmodified parental plant (morphological characteristics, physiological activities, growth characteristics, etc.); a detailed description of the purpose for the field test including a detailed description of the field test design; the proposed maximum size of the field test; the proposed earliest date of the field planting and the length of time requested for the permit (the length of time that the field test will exist); the proposed number of plantings; a detailed description of the field trial location; a detailed description of the procedures that will be used to prevent the release or movement of the regulated article outside of the test area (this includes monitoring for the regulated article at the end of the field test); and a detailed description of the proposed method of final disposition of the regulated article. For full details in applying for a permit, check the BRS website (http://www.aphis.usda.gov/permits/brs_epermits.shtml).

With the advent of genetically engineered perennial grasses and the availability of single herbicide tolerance genes and other easily detectable molecular markers, extremely precise information is becoming available on pollen flow for perennial grasses. These data are generally much more precise than the data available based on morphological characteristics of which the precision may be as small as 0.1% (parts per thousand), whereas the data from single herbicide tolerance genes or molecular markers may be as precise as 0.0001% (parts per million). With additional data becoming available on pollen flow for creeping bentgrass (Wipff and Fricker, 2001; Wipff, 2002; Belanger *et al.*, 2003; Watrud *et al.*, 2004; Petition 03-104-01p), tall fescue (Wang *et al.*, 2004), and Kentucky bluegrass (Johnson *et al.*, 2006) and on possible creeping bentgrass seed movement (Reichman *et al.*, 2006), APHIS is updating its guidance for field testing of wind-pollinated perennial grasses. These recent studies have had very similar results. As with most plants, cross-pollination with other plants is most likely between sexually compatible plants that are closest to each other. With wind pollination, the maximum distance that viable pollen may pollinate sexually compatible plants is highly dependent on the speed and direction of air movement. In addition, the amount of viable pollen produced has an effect on the farthest distance that one or more successful pollinations will most likely take place. In all of the pollination studies cited above, only Watrud *et al.* (2004) studied a creeping bentgrass pollen source that greatly exceeded 500 pollen source plants with that study measuring pollen flow from 162 ha (400 acres) with a successful cross-pollination documented at 21 km. The greatest distance found for a successful cross-pollination with one of the smaller studies (Wipff, 2002) was a distance of 426 m with a pollen source of 298 plants.

Perennial grass species used for turf and forage have many characteristics in common. They generally are perennial, wind pollinated, small seeded, easily propagated vegetatively, spread vegetatively by stolons and/or rhizomes (and to some extent by tillers), tolerant of traffic, mowing, grazing, and burning, and tolerant of cold and/or heat stress by becoming dormant. Many of the perennial grass species have some seed dormancy, and many are easily propagated by seeds. These same characteristics are typical of perennial grass species used for forages. In fact, many turfgrass species are used for forage as well as for turf. For the species cited above in the pollen flow studies, creeping bentgrass is used mainly for turf purposes but it has been used frequently for forage

purposes, tall fescue is used for forage and turf, and Kentucky bluegrass is used mainly for turf and is frequently found in pastures and other grazed areas.

All of these characteristics of perennial grasses need to be taken into account when considering confinement procedures for field testing GE turfgrasses. Confinement procedures need to take into consideration any means of gene flow—pollen, seed, and vegetative parts. Testing of turfgrasses is generally conducted in two distinct phases—turf performance and seed production.

When testing its turf performance, it is mowed one to several times a week resulting in the removal of all or most of its flowers. If this is the case, any concerns about pollen flow or seed movement are completely eliminated or greatly reduced, but concerns about vegetative movement and volunteer plants after the removal of the test would still need to be addressed. Various procedures can possibly be used to assure confinement: a nontransgenic border area or fallow zone surrounding the test to allow personnel to identify and control any transgenic plants that may be growing outside of the test; all equipment used in the test area is cleaned inside the test plot or border area before it is moved off the test site; access to the site is restricted to authorized personnel who have been trained to follow suitable procedures to minimize the spread of the regulated article; in addition to the border area, an additional isolation area surrounding the test area which is monitored for the presence of sexually compatible plants (plants that have the potential to cross with the regulated article and produce viable offspring) and if present are destroyed or prevented from flowering; and upon termination of the trial, monitor the regulated trial area for volunteers for an agreed period of time. For clarification, the regulated trial area includes the test area and the surrounding border area.

For testing during the seed production phase, pollen flow and seed movement need to be addressed as well. Possible methods for limiting pollen flow include: using sterile plants that will not produce pollen or seed; using male sterile plants; bagging the flowers/seed heads; covering all plants with a tight mesh netting that will restrict pollen flow; growing plants in an isolated area where no sexually compatible plants are growing in the immediate area—this includes seed fields of the same or related species, feral plants of the same or related species, and plants of "wild" related species; and restricting access to the site to authorized personnel who have been trained to follow suitable procedures to minimize the spread of pollen on clothes and equipment. For growing the flowering plants in isolation to be effective, scouting for sexually compatible plants at the future site of a proposed trial a year before installing the test is helpful, and then monitoring for sexually compatible plants in the isolation area during the flowering period of the trial itself shortly before or in the early flowering period and destroying any plants found during these monitoring periods would be required.

Possible methods for limiting seed movements include: using sterile plants that will not produce seed; bagging the flowers/seed heads; covering all plants with netting that will restrict seed movement by wind or birds; placing fence around the trial that would restrict animals and unauthorized individuals from walking across the trial; placing harvested seed heads directly into a container instead of laying seed heads down to dry; using dedicated equipment to avoid mixing regulated article with unregulated material; restricting access to the site to authorized personnel who have been trained to follow suitable procedures to minimize the spread of seed on clothes and equipment; conducting the trial in an inhospitable environment so escapes are unlikely to establish and feral populations are unlikely to exist; using a nontransgenic border area or fallow zone surrounding the test to allow personnel to identify and control any transgenic plants that may be growing outside of the test—the border will also provide an area to service and clean equipment, and for a staging area for placing seed prior to planting or placing harvest containers; having a substantial isolation area in addition to the border between the trial and any harvested crop, especially for any seed crop of perennial grasses; and upon termination of the trial, monitoring the regulated trial area for volunteers for an agreed period of time. Again, the regulated trial area includes the test area and the surrounding border area.

All of the confinement measures mentioned above are possible suggestions to consider when

planning to install and conduct trials of transgenic turfgrasses. But for each species, possibly the transgenic characteristic, and the size of the trial, the confinement measures may vary. The length of any recommended monitoring period depends heavily on the seed dormancy characteristics of each species. Therefore decisions on the specific requirements need to be made on a case-by-case basis.

3.2 Obtaining Nonregulated Status and Commercialization of Transgenic Turfgrasses

As with any variety development program, a point is reached where the performance data obtained in various trials appear to justify a commercial release of the developed variety. However, unlike a conventionally developed variety in which no genetic engineering techniques were used, in addition to convincing only the potential buyers or customers of the new variety's advantages, three different government agencies may need to be convinced that it is safe to consume as food or feed (FDA), it has no pesticidal properties that make it harmful to the environment, unsafe to consume, or requires a quantity of a pesticide that is considered to be detrimental to the environment (EPA), and/or it is not detrimental to agriculture or the environment (USDA). For the USDA, a person needs to submit a petition for determination of nonregulated status. If approved, the new variety is considered deregulated, and the product and its progeny would no longer require USDA-APHIS review for movement or release in the United States.

In this petition, the developer should include the following: the purpose or rationale for the petition; a description of the biology of the plant before it was genetically engineered; description of the transformation system; a detailed description of the donor gene(s), other DNA sequences and its products along with the map of the construct used in the transformation; molecular characterization analysis of the DNA insert in the recipient (insert length, number, and partial pieces of the construct, presence of the plasmid backbone, and stability of the insert); a detailed breeding diagram of the selection practices and procedures used to develop each generation, and population of plants used to provide data in the petition; inheritance of the transgenic trait; differences between the GE plant and the original plant; a description or rationale for the use of the control varieties or lines used in the comparison of the regulated article to the unmodified recipient (this may be the same line as the regulated article without the transgene and/or an array of varieties representative of the species); detailed phenotypic comparisons of the transgenic plant and unmodified recipient for various characteristics—seed germination, seed vigor, seed establishment, seed dispersal, seed dormancy, seed yield, time and length of flowering and seed production period, growth habit, vegetative vigor (horizontal spread, plant height, and/or biomass), tolerance to biotic and abiotic stresses, outcrossing frequency, effect on pollinator species, pollen characteristics (size, shape, amount, stickiness, longevity, etc.), symbionts, asexual reproduction (vegetative and seed), and self-compatibility (http://www.aphis.usda.gov/brs/canadian/appe nannex2e.pdf, http://www.cast-science.org/ websiteuploads/pdfs/turfbiotech_is.pdf).

The petitioner may address each of the above topics using the scientific literature and/or test results developed specifically for the petition. These measurements or observations on all of the phenotypic characteristics are useful in the review of the petition for determining the existence of "unintended effects". From a variety development perspective, almost all of the phenotypic characteristics are noted during the variety testing stages anyway so the documentation of these observations requires only a small additional effort if the tests are well planned in advance of the petition.

All of the field tests conducted under APHIS regulation for this regulated product need to be listed in the petition. All required field-test reports for each of the field tests need to be completed and on file with USDA-APHIS. The petitioner is also required to provide any known information that indicates that the regulated article may pose a greater plant pest risk than that of the unmodified recipient plant.

After reviewing the petition, BRS conducts an environmental assessment in compliance with the National Environmental Policy Act to analyze the potential impacts the GE organism may have on agriculture and the environment. This assessment

includes a wide variety of environmental parameters and looks for possible effects to threatened and endangered species. After the completion of the assessment by BRS, it is posted on the Web along with a copy of the developer's petition (with all confidential business information deleted) and made available for a 60-day public comment period through the *Federal Register* announcement. Following these procedures, BRS then decides to approve or deny the petition. For obtaining details on completing the petition, guidance is provided on the BRS website (http://www.aphis.usda.gov/biotechnology/brs_main.shtml) indicating the type of molecular details and the agronomic data that are helpful in evaluating the petition. The past petitions approved by BRS are also available through the BRS website.

4. FUTURE PERSPECTIVES

The rapid development of turfgrass genetic transformation technologies has made it possible to apply biotechnological approaches for trait modifications in a large number of grass species. The many promising data obtained so far point to the great potential of commercialization of new turfgrass cultivars with genetically improved traits. However, concerns on transgene flow from genetically modified plants to compatible wild species and unintended ecological consequences of potential transgene introgression impose hurdles for the deregulation of any transgenic perennial species. Development and implementation of molecular strategies for gene containment in genetically modified transgenic plants with improved performance may facilitate public acceptance and commercialization of GM products in turfgrass species. Besides the use of strategies involving manipulation of genes that control plant reproductive growth for gene containment, chloroplast genetic engineering to promote maternal inheritance of transgenes is a promising option for prevention of gene flow through pollen dispersion. Maternal inheritance of cytoplasmic organelles is shared by plant (chloroplast) and animal (mitochondria) systems. The prevalent pattern of plastid inheritance found in majority of angiosperms is uniparental–maternal and chloroplast genomes are maternally inherited in most crops (Daniell, 2002). Maternal inheritance of transgenes and prevention of gene flow through pollen in chloroplast transgenic plants have been successfully demonstrated in several plant species, including tobacco and tomato (Daniell *et al.*, 1998; Ruf *et al.*, 2001). In addition to its potential for gene containment, chloroplast engineering offers several other advantages, including a high level of transgene expression, multigene engineering in a single transformation event, lack of gene silencing, position effect, pleiotropic effects, and undesirable foreign DNA. Chloroplast engineering also can greatly enhance the capability of some turfgrass species as potential bioreactors for large-scale production of industrial enzymes, biodegradable plastics, pharmaceuticals, vaccines, and antibodies. With the success of chloroplast engineering in the first monocot species, rice (Lee *et al.*, 2006), and the availability of creeping bentgrass chloroplast genome sequence, the very first in turfgrass species (Saski *et al.*, 2007), chloroplast engineering in turfgrass species could be expected in the very near future.

Although genomics studies in turfgrass species lag behind that of other agriculturally important food grasses due to the complex genetic constituents of turf species, and the lack of substantial public funding for support, data generated from major crop plants (rice, wheat, and maize), especially the completion of the genome sequencing of the first grass species, rice, provide invaluable information in developing molecular and genomics tools for gene discoveries and for better understanding of molecular mechanisms defining plant resistance to biotic and abiotic stresses as well as other biological processes. This will result in more effective tools and strategies in genetically improving turfgrass for enhanced stress tolerance and desired plant morphologies using biotechnological approaches.

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REFERENCES

Adam, D. (2002) Hostilities resume over future of GM crops. *Nature* **419**, 327.

Ahn, B.J., Huang, F.H. and King, J.W. (1985) Plant regeneration through somatic embryogenesis in common bermudagrass tissue-culture. *Crop Science* **25**, 1107–1109.

Ahn, B.J., Huang, F.H. and King, J.W. (1987) Regeneration of bermudagrass cultivars and evidence of somatic embryogenesis. *Crop Science* **27**, 594–597.

Akiyoshi, D.E., Morris, R.O., Hinr, R., Mischke, B.S., Kosuge, T., Garfinkel, D., Gordon, M.P. and Nester, E.W. (1983) Cytokinin auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. *Proceedings of the National Academy of Sciences of the USA* **80**, 407–411.

Albert, H., Dale, E.C., Lee, E. and Ow, D.W. (1995) Site-specific integration of DNA into wild-type and mutant lox sites placed in the plant genome. *Plant Journal* **7**, 649–659.

Alkhayri, J.M., Huang, F.H., Thompson, L.F. and King, J.W. (1989) Plant regeneration of zoysiagrass from embryo-derived callus. *Crop Science* **29**, 1324–1325.

Altpeter, F. and Posselt, U.K. (2000) Improved plant regeneration from cell suspensions of commercial cultivars, breeding- and inbred lines of perennial ryegrass (*Lolium perenne* L.). *Journal of Plant Physiology* **156**, 790–796.

Altpeter, F. and Xu, J.P. (2000) Rapid production of transgenic turfgrass (*Festuca rubra* L.) plants. *Journal of Plant Physiology* **157**, 441–448.

Altpeter, F., Xu, J.P. and Ahmed, S. (2000) Generation of large numbers of independently transformed fertile perennial ryegrass (*Lolium perenne* L.) plants of forage- and turf-type cultivars. *Molecular Breeding* **6**, 519–528.

Apse, M.P., Aharon, G.S., Snedden, W.A. and Blumwald, E. (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. *Science* **285**, 1256–1258.

Artunduaga, I.R., Taliaferro, C.M. and Johnson, B.B. (1989) Induction and growth of callus from immature inflorescences of Zebra bermudagrass as affected by casein hydrolysate and 2,4-D concentration. *In Vitro Cellular and Developmental Biology—Plant* **25**, 753–756.

Artunduaga, I.R., Taliaferro, C.M. and Johnson, B.L. (1988) Effects of auxin concentration on induction and growth of embryogenic callus from young inflorescence explants of old-world bluestem (*Bothriochloa* spp.) and bermuda (*Cynodon* spp.) grasses. *Plant Cell, Tissue and Organ Culture* **12**, 13–19.

Arumuganathan, K. and Earle, E.D. (1991) Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter* **9**, 221–231.

Arumuganathan, K., Tallury, S.P., Fraser, M.L., Bruneau, A.H. and Qu, R. (1999) Nuclear DNA content of thirteen turfgrass species by flow cytometry. *Crop Science* **39**, 1518–1521.

Asano, Y. (1989) Somatic embryogenesis and protoplast culture in Japanese lawngrass (*Zoysia japonica*). *Plant Cell Reports* **8**, 141–143.

Asano, Y., Ito, Y., Fukami, M., Sugiura, K. and Fujiie, A. (1998) Herbicide-resistant transgenic creeping bentgrass plants obtained by electroporation using an altered buffer. *Plant Cell Reports* **17**, 963–967.

Asano, Y., Katsumoto, H., Inokuma, C., Kaneko, S., Ito, Y. and Fujiie, A. (1996) Cytokinin and thiamine requirements and stimulative effects of riboflavin and alpha-ketoglutaric acid on embryogenic callus induction from the seeds of *Zoysia japonica* Steud. *Journal of Plant Physiology* **149**, 413–417.

Aswath, C.R., Kim, S.H., Mo, S.Y. and Kim, D.H. (2005) Transgenic plants of creeping bent grass harboring the stress inducible gene, *9-cis-epoxycarotenoid dioxygenase*, are highly tolerant to drought and NaCl stress. *Plant Growth Regulation* **47**, 129–139.

Austin, S., Ziese, M. and Sternberg, N. (1981) A novel role of site-specific recombination in maintenance of bacterial replicons. *Cell* **25**, 729–736.

Bai, Y. (2001) Tissue culture and genetic transformation of Tall Fescue. Ph.D. Dissertation, North Carolina State University, USA.

Bai, Y. and Qu, R. (2000) An evaluation of callus induction and plant regeneration in twenty-five turf-type tall fescue (*Festuca arundinacea* Schreb.) cultivars. *Grass and Forage Science* **55**, 326–330.

Bai, Y. and Qu, R. (2001a) Factors influencing tissue culture responses of mature seeds and immature embryos in turf-type tall fescue. *Plant Breeding* **120**, 239–242.

Bai, Y. and Qu, R. (2001b) Genetic transformation of elite turf-type cultivars of tall fescue. *International Turfgrass Society Research Journal* **9**, 129–136.

Bajaj, S., Ran, Y.D., Phillips, J., Kularajathevan, G., Pal, S., Cohen, D., Elborough, K. and Puthigae, S. (2006) A high throughput *Agrobacterium tumefaciens*-mediated transformation method for functional genomics of perennial ryegrass (*Lolium perenne* L.). *Plant Cell Reports* **25**, 651–659.

Barbieri, L., Battelli, M.G. and Stripe, F. (1993) Ribosome-inactivating proteins from plants. *Biochimica et Biophysica Acta* **1154**, 237–282.

Baulcombe, D. (2005) RNA silencing. *Trends in Biochemical Sciences* **30**, 290–293.

Belanger, F.C., Meagher, T.R., Day, P.R., Plumley, K.A. and Meyer, W.A. (2003) Interspecific hybridization between *Agrostis stolonifera* and related *Agrostis* species under field conditions. *Crop Science* **43**, 240–246.

Bettany, A.J.E., Dalton, S.J., Timms, E., Dhanoa, M.S. and Morris, P. (2002) Effect of selectable gene to reporter gene ratio on the frequency of co-transformation and co-expression of *uidA* and hpt transgenes in protoplast-derived plants of tall fescue. *Plant Cell, Tissue and Organ Culture* **68**, 177–186.

Bettany, A.J.E., Dalton, S.J., Timms, E., Manderyck, B., Dhanoa, M.S. and Morris, P. (2003) *Agrobacterium tumefaciens*-mediated transformation of *Festuca arundinacea* (Schreb.) and *Lolium multiflorum* (Lam.). *Plant Cell Reports* **21**, 437–444.

Bettany, A.J.E., Dalton, S.J., Timms, E. and Morris, P. (1998) Stability of transgene expression during vegetative propagation of protoplast-derived tall fescue (*Festuca arundinacea* Schreb.) plants. *Journal of Experimental Botany* **49**, 1797–1804.

Bhalla, P.L., Swoboda, I. and Singh, M.B. (1999) Antisense-mediated silencing of a gene encoding a major ryegrass pollen allergen. *Proceedings of the National Academy of Sciences of the USA* **96**, 11676–11680.

Blanche, F.C., Krans, J.V. and Coats, G.E. (1986) Improvement in callus growth and plantlet formation in creeping bentgrass. *Crop Science* **26**, 1245–1248.

Bohnert, H.J., Nelson, D.E. and Jensen, R.G. (1995) Adaptations to environmental stresses. *Plant Cell* **7**, 1099–1111.

Bonos, S.A., Plumley, K.A. and Meyer, W.A. (2002) Ploidy determination in *Agrostis* using flow cytometry and morphological traits. *Crop Science* **42**, 192–196.

Boyd, L.A. and Dale, P.J. (1986) Callus production and plant regeneration from mature embryos of *Poa pratensis* L. *Plant Breeding* **97**, 246–254.

Bradley, D.E., Bruneau, A.H. and Qu, R. (2001) Effects of cultivar, explant treatment, and medium supplements on callus induction and plantlet regeneration in perennial ryegrass. *International Turfgrass Society Research Journal* **9**, 152–156.

Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. (2000) Responses to abiotic stresses. In: Buchanan, B., Gruissem, W. and Jones, R. (eds.) *Biochemistry and Molecular Biology of Plants*. American Society of Plant Biologists, Rockville, pp. 1158–1203.

Broach, J.R., Guarascio, V.R. and Jayaram, M. (1982) Recombination within the yeast plasmid 2 micron circle is site specific. *Cell* **29**, 227–234.

Brown, D.C.W. and Thorpe, T.A. (1995) Crop improvement through tissue-culture. *World Journal of Microbiology and Biotechnology* **11**, 409–415.

Bürglin, T.R. (1997) Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Research* **25**, 4173–4180.

Burton, G.W. (1964) Tifgreen (Tif328) bermudagrass for golf greens. *USGA Green Section Record* **2**, 11–13.

Burton, G.W. (1966) Tifway (Tif419) bermudagrass. *Crop Science* **6**, 93–94.

Busey, P. (2003) St. Augustinegrass. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 309–330.

Cao, M.X., Huang, J.Q., He, Y.L., Liu, S.J., Wang, C.L., Jiang, W.Z. and Wei, Z.M. (2006) Transformation of recalcitrant turfgrass cultivars through improvement of tissue culture and selection regime. *Plant Cell, Tissue and Organ Culture* **85**, 307–316.

Cardona, C.A. and Duncan, R.R. (1997) Callus induction and high efficiency plant regeneration via somatic embryogenesis in paspalum. *Crop Science* **37**, 1297–1302.

Casler, M.D. and Duncan, R.R. (2003) Origins of the turfgrasses. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 5–23.

Chai, B., Maqbool, S.B., Hajela, R.K., Green, D., Vargas Jr, J.M., Warkentin, D., Sabzikar, R. and Sticklen, M.B. (2002) Cloning of a chitinase-like cDNA (*hs2*), its transfer to creeping bentgrass (*Agrostis palustris* Huds.) and development of brown patch (*R. solani*) disease resistant transgenic lines. *Plant Science* **163**, 183–193.

Chai, B.C. and Sticklen, M.B. (1998) Application of biotechnology in turfgrass genetic improvement. *Crop Science* **38**, 1320–1338.

Chai, B.F., Liang, A.H., Wang, W. and Wei, H. (2003) *Agrobacterium*-mediated transformation of Kentucky bluegrass. *Acta Botanica Sinica* **45**, 966–973.

Chai, M.L., Senthil, K.K. and Kim, D.H. (2004) Transgenic plants of colonial bentgrass from embryogenic callus via Agrobacterium-mediated transformation. *Plant Cell, Tissue and Organ Culture* **77**, 165–171.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. and Prasher, D.C. (1994) Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805.

Chatterton, N.J., Harrison, P.A., Bennett, J.H. and Asay, K.H. (1989) Carbohydrate partitioning in 185 accessions of *Gramineae* under warm and cool temperatures. *Journal of Plant Physiology* **134**, 169–179.

Chaudhury, A. and Qu, R. (2000) Somatic embryogenesis and plant regeneration of turf-type bermudagrass: Effect of 6-benzyladenine in callus induction medium. *Plant Cell, Tissue and Organ Culture* **60**, 113–120.

Chen, X., Yang, W.Q., Sivamani, E., Bruneau, A.H., Wang, B.H. and Qu, R. (2005) Selective elimination of perennial ryegrass by activation of a proherbicide through engineering *E. coli argE* gene. *Molecular Breeding* **15**, 339–347.

Cho, M.J., Ha, C.D. and Lemaux, P.G. (2000) Production of transgenic tall fescue and red fescue plants by particle bombardment of mature seed-derived highly regenerative tissues. *Plant Cell Reports* **19**, 1084–1089.

Chu, C.C., Wang, C.C., Sun, C.S., Hsu, C., Yin, K.C., Chu, C.Y. and Bi, F.Y. (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Scientia Sinica* **18**, 659–668.

Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant Journal* **16**, 735–743.

Coen, E.S., Romero, J.M., Doyle, S., Elliott, R., Murphy, G. and Carpenter, R. (1990) *Floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. *Cell* **63**, 1311–1322.

Collinge, D.B., Kragh, K.M., Mikkelsen, J.D., Nielsen, K.K., Rasmussen, U. and Vad, K. (1993) Plant chitinase. *Plant Journal* **3**, 31–40.

Creemers-Molenaar, J., Van Der Valk, P., Loeffen, J.P.M. and Zaal, M. (1989) Plant regeneration from suspension-cultures and protoplasts of *Lolium perenne* L. *Plant Science* **63**, 167–176.

Creemers-Molenaar, J., Vaneeuwijk, F.A. and Krens, F.A. (1992) Culture optimization for perennial ryegrass protoplasts. *Journal of Plant Physiology* **139**, 303–308.

Crozier, A., Kamiya, Y., Bishop, G. and Yokota, T. (2000) Biosynthesis of hormones and elicitor molecules. In: Buchanan, B., Gruissem, W. and Jones, R. (eds.) *Biochemistry and Molecular Biology of Plants*. American Society of Plant Biologists, Rockville, pp. 850–929.

Dai, W.D., Bonos, S., Guo, Z. Meyer, W.A., Day, P.R. and Belanger, F.C. (2003) Expression of pokeweed antiviral proteins in creeping bentgrass. *Plant Cell Reports* **21**, 497–502.

Dale, P.J., Clarke, B. and Fontes, E.M.G. (2002) Potential for the environmental impact of transgenic crops. *Nature Biotechnology* **20**, 567–574.

Dale, P.J. and Dalton, S.J. (1983) Immature inflorescence culture in *Lolium, Festuca, Phleum and Dactylis*. *Zeitschrift für Pflanzenphysiologie* **111**, 39–45.

Dalton, S.J. (1988) Plant regeneration from cell suspension protoplasts of *Festuca arundinacea* Schreb. (tall fescue) and *Lolium perenne* L. (perennial ryegrass). *Journal of Plant Physiology* **132**, 170–175.

Dalton, S.J., Bettany, A.J.E., Timms, E. and Morris, P. (1995) The effect of selection pressure on transformation frequency and copy number in transgenic plants of tall fescue (*Festuca arundinacea* Schreb.). *Plant Science* **108**, 63–70.

Dalton, S.J., Bettany, A.J.E., Timms, E. and Morris, P. (1998) Transgenic plants of *Lolium multiflorum*, *Lolium perenne*, *Festuca arundinacea* and *Agrostis stolonifera* by silicon carbide fibre-mediated transformation of cell suspension cultures. *Plant Science* **132**, 31–43.

Dalton, S.J., Bettany, A.J.E., Timms, E. and Morris, P. (1999) Co-transformed, diploid *Lolium perenne* (perennial ryegrass), *Lolium multiflorum* (Italian ryegrass) and *Lolium temulentum* (darnel) plants produced by microprojectile bombardment. *Plant Cell Reports* **18**, 721–726.

Dangl, J.L., Dietrich, R.A. and Richberg, M.H. (1996) Death don't have no mercy: cell death programs in plant–microbe interactions. *Plant Cell* **8**, 1793–1807.

Daniell, H. (2002) Molecular strategies for gene containment in transgenic crops. *Nature Biotechnology* **20**, 581–586.

Daniell, H., Datta, R., Varma, S., Gray, S. and Lee, S.B. (1998) Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnology* **16**, 345–348.

De Block, M., Botterman, J., Vandewiele, M., Dockx, J., Thoen, C., Gosselé Movva, N.R., Thompson, C., Van Montagu, M. and Leemans, J. (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO Journal* **6**, 2513–2518.

De Lucca, A.J., Cleveland, T.E. and Wedge, D.E. (2005) Plant-derived antifungal proteins and peptides. *Canadian Journal of Microbiology* **51**, 1001–1014.

Dong, S.J. and Qu, R. (2005) High efficiency transformation of tall fescue with *Agrobacterium tumefaciens*. *Plant Science* **168**, 1453–1458.

Dong, S.J., Shew, H.D., Tredway, L.P., Lu, J., Sivamani, E., Miller, E.S. and Qu, R. (2008) Expression of the bacteriophage T4 lysozyme gene in tall fescue confers resistance to gray leaf spot and brown patch diseases. *Transgenic Research* **17**, 47–57.

Dong, S.J., Tredway, L.P., Wang, G.-L., Sivamani, E. and Qu, R. (2007) Resistance of transgenic tall fescue to two major fungal diseases. *Plant Science* **173**, 501–509.

Duncan, R.R. (2003) Seashore Paspalum (*Paspalum vaginatum* Swartz). In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 295–307.

Düring, K., Porsch, P., Mahn, A., Brinkmann, O. and Gieffers, W. (1999) The non-enzymatic microbicidal activity of lysozymes. *FEBS Letters* **449**, 93–100.

Ebskamp, M.J., Van Der Meer, I.M., Spronk, B.A., Weisbeek, P.J. and Smeekens, S.C.M. (1994) Accumulation of fructose polymers in transgenic tobacco. *Bio/Technology* **12**, 272–275.

Edens, L., Heslinga, L., Klock, R., Ledeboer, A.M., Maat, J., Toonen, M.Y., Visser, C. and Verrips, C.T. (1982) Cloning of cDNA encoding the sweet-tasting plant protein thaumatin and its expression in *Escherichia coli*. *Gene* **18**, 1–12.

Eizenga, G.C. and Dahleen, L.S. (1990) Callus production, regeneration and evaluation of plants from cultured inflorescences of tall fescue (*Festuca arundinacea* Schreb.). *Plant Cell, Tissue and Organ Culture* **22**, 7–15.

Emmons, R. (1995) Turfgrass Science and Management, 2nd edn. Delmar, New York.

Endo, Y. and Wool, I.G. (1982) The site of action of α-sarcin cleavage site in 28S ribosomal ribonucleic acid. *Journal of Biological Chemistry* **257**, 9054–9060.

Engelke, M.C. and Anderson, S. (2003) Zoysiagrass (*Zoysia* spp.). In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 271–285.

Faiss, M., Zalubilova, J., Strnad, M. and Schmulling, T. (1997) Conditional transgenic expression of the *ipt* gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. *Plant Journal* **12**, 401–415.

Fei, S., Read, P.E. and Riordan, T.P. (1997) *In vitro* regeneration of *Buchloe dactyloides* (Nutt.) Englem. through immature inforescence culture. *International Turfgrass Society Research Journal* **8**, 283–289.

Fei, S., Read, P.E. and Riordan, T.P. (2000) Improvement of embryogenic callus induction and shoot regeneration of buffalograss by silver nitrate. *Plant Cell, Tissue and Organ Culture* **60**, 197–203.

Fei, S., Bishnoi, U.S., Riordan, T. and Read, P. (2001) Embryogenic callus induction and plant regeneration of buffalograss through leaf base and seedling segment culture. *International Turfgrass Society Research Journal* **9**, 162–164.

Fei, S. and Nelson, E. (2004) Greenhouse evaluation of fitness-related reproductive traits in Roundup®-tolerant transgenic creeping bentgrass (*Agrostis stolonifera* L.). *In Vitro Cellular and Developmental Biology—Plant* **40**, 266–273.

Fei, S., Riordan, T., Bishnoi, U. and Clemente, T. (2005) Regeneration of transgenic buffalograss (*Buchloe dactyloides*) with glyphosate resistance using particle bombardment. *International Turfgrass Society Research Journal* **10**, 550–554.

Fei, S., Riordan, T. and Read, P. (2002) Stepwise decrease of 2, 4-D and addition of BA in subculture medium stimulated shoot regeneration and somatic embryogenesis in buffalograss. *Plant Cell, Tissue and Organ Culture* **70**, 275–279.

Folling, M., Pedersen, C. and Olesen, A. (1998) Reduction of nuclease activity from *Lolium* protoplasts: effect on transformation frequency. *Plant Science* **139**, 29–40.

Fu, D., Tisserat, N.A., Xiao, Y., Settleb, D., Muthukrishnan, S. and Liang, G.H. (2005) Overexpression of rice TLPD34 enhances dollar-spot resistance in transgenic bentgrass. *Plant Science* **168**, 671–680.

Fu, D.L., St Amand, P.C., Xiao, Y.M., Muthukrishnan, S. and Liang, G.H. (2006) Characterization of T-DNA integration in creeping bentgrass. *Plant Science* **170**, 225–237.

Fukuda, A., Nakamura, A., Tagiri, A., Tanaka, H., Miyao, A., Hirochika, H. and Tanaka, Y. (2004) Function, intracellular localization and the importance in salt tolerance of a vacuolar Na^+/H^+ antiporter from rice. *Plant and Cell Physiology* **45**, 146–159.

Fukuda, A., Nakamura, A. and Tanaka, Y. (1999) Molecular cloning and expression of the Na^+/H^+ exchanger gene in *Oryza sativa*. *Biochimica et Biophysica Acta* **1446**, 149–155.

Gan, S. and Amasino, R.M. (1997) Making sense of senescence: molecular genetic regulation and manipulation of leaf senescence. *Plant Physiology* **113**, 313–319.

Gao, C.X., Jiang, L., Folling, M., Han, L.B. and Nielsen, K.K. (2006) Generation of large numbers of transgenic Kentucky bluegrass (*Poa pratensis* L.) plants following biolistic gene transfer. *Plant Cell Reports* **25**, 19–25.

Garcia, A., Dalton, S.J. and Humphreys, M.O. (1994) Reproductive disturbances and phosphoglucoisomerase instability in *Festuca arundinacea* (tall fescue) plants regenerated from callus and cell-suspension cultures. *Heredity* **73**, 355–362.

Gardner, D.S., Danneberger, T.K., Nelson, E., Meyer, W. and Plumley, K. (2003) Relative fitness of glyphosate-resistant creeping bentgrass lines in Kentucky bluegrass. *HortScience* **38**, 455–459.

Gardner, D.S., Danneberger, T.K. and Nelson, E.K. (2004) Lateral spread of glyphosate-resistant transgenic creeping bentgrass (*Agrostis stolonifera*) lines in established turfgrass swards. *Weed Technology* **18**, 773–778.

Gaxiola, R., Li, J., Undurraga, S., Dang, L.M., Allen, G.J., Alper, S. and Fink, G.R. (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H^+-pump. *Proceedings of the Naional Academy of Sciences of the USA* **98**, 11444–11449.

Ge, Y.X., Norton, T. and Wang, Z.Y. (2006) Transgenic zoysiagrass (*Zoysia japonica*) plants obtained by *Agrobacterium*-mediated transformation. *Plant Cell Reports* **25**, 792–798.

Goldman, J.J., Hanna, W.W., Fleming, G.H. and Ozias-Akins, P. (2004a) Ploidy variation among herbicide-resistant bermudagrass plants of cv. TifEagle transformed with the *bar* gene. *Plant Cell Reports* **22**, 553–560.

Goldman, J.J., Hanna, W.W. and Ozias-Akins, P. (2004b) Plant regeneration and an analysis of somaclonal variation from TifEagle and TifSport bermudagrass cultivars. *HortScience* **39**, 1381–1384.

Graham, L.S. and Sticklen, M.B. (1994) Plant chitinase. *Canadian Journal of Botany* **72**, 1057–1083.

Griffin, J.D. and Dibble, M.S. (1995) High-frequency plant-regeneration from seed-derived callus-cultures of Kentucky bluegrass (*Poa pratensis* L). *Plant Cell Reports* **14**, 721–724.

Gritz, L. and Davies, J. (1983) Plasmid-encoded hygromycin-B resistance—the sequence of hygromycin-B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. *Gene* **25**, 179–188.

Guo, Z., Bonos, S., Meyer, W.A., Day, P.R. and Belanger, F.C. (2003) Transgenic creeping bentgrass with delayed dollar spot symptoms. *Molecular Breeding* **11**, 95–101.

Ha, C.D., Lemaux, P.G. and Cho, M.J. (2001) Stable transformation of a recalcitrant Kentucky bluegrass (*Poa pratensis* L.) cultivar using mature seed-derived highly regenerative tissues. *In Vitro Cellular and Developmental Biology—Plant* **37**, 6–11.

Ha, S.B., Wu, F.S. and Thorne, T.K. (1992) Transgenic turf-type tall fescue (*Festuca arundinacea* Schreb.) plants regenerated from protoplasts. *Plant Cell Reports* **11**, 601–604.

Hammond-Kosack, K.E. and Jones, J.D.G. (1997) Plant disease resistance gene. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 575–607.

Han, N., Chen, D., Bian, H.W., Deng, M.J. and Zhu, M.Y. (2005) Production of transgenic creeping bentgrass *Agrostis stolonifera* var. palustris plants by *Agrobacterium tumefaciens*-mediated transformation using hygromycin selection. *Plant Cell, Tissue and Organ Culture* **81**, 131–138.

Hanna, W.W., Burton, G.W. and Johnson, A.W. (1990) Registration of Tifton 10 turf bermudagrass. *Crop Science* **30**, 1355–1356.

Hanna, W.W., Carrow, R.N. and Powell, A.J. (1997) Registration of 'Tift 94' bermudagrass. *Crop Science* **37**, 1012.

Hanna, W.W. and Elsner, J.E. (1999) Registration of 'TifEagle' bermudagrass. *Crop Science* **39**, 1258.

Hanna, W.W. and Liu, J. (2003) Centipedegrass (*Eremochloa ophiuroides*). In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 287–293.

Hartley, R.W. (1988) Barnase and Barstar: expression of its cloned inhibitor permits expression of a cloned ribonuclease. *Journal of Molecular Biology* **202**, 913–915.

Hartman, C.L., Lee, L., Day, P.R. and Tumer, N.E. (1994) Herbicide-resistant turfgrass (*Agrostis palustris* Huds.) by biolistic transformation. *Bio/Technology* **12**, 919–923.

Hattori, T., Nakagava, T., Maeshma, M., Nakamura, K. and Asahi, T. (1985) Molecular cloning and nucleotide sequence of the cDNA for sporamin, the major soluble protein of sweet potato tuberous roots. *Plant Molecular Biology* **5**, 315–320.

Hendry, G.A.F. (1993) Evolutionary origins and natural functions of fructans—a climatological, biogeographic and mechanistic appraisal. *New Phytologist* **123**, 3–14.

Hewelt, A., Prinsen, E., Schell, J., Van Onckelen, H. and Schmulling, T. (1994) Promoter tagging with a promoterless *ipt* gene leads to cytokinin-induced phenotypic variability in transgenic tobacco plants: implications of gene dosage effects. *Plant Journal* **6**, 879–891.

Hisano, H., Kanazawa, A., Kawakami, A., Yoshida, M. Shimamotoa, Y. and Yamada, T. (2004) Transgenic perennial ryegrass plants expressing wheat fructosyltransferase genes accumulate increased amounts of fructan and acquire increased tolerance on a cellular level to freezing. *Plant Science* **167**, 861–868.

Hsieh, T.H., Lee, J.T., Charng, Y.Y. and Chan, M.T. (2002a) Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. *Plant Physiology* **130**, 618–626.

Hsieh, T.H., Lee, J.T., Yang, P.T., Chiu, L.H., Charng, Y.Y., Wang, Y.C. and Chan, M.T. (2002b) Heterology expression of the *Arabidopsis* C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. *Plant Physiology* **129**, 1086–1094.

Hu, Y., Jia, W., Wang, J., Zhang, Y., Yang, L. and Lin, Z (2005) Transgenic tall fescue containing the *Agrobacterium tumefaciens ipt* gene shows enhanced cold tolerance. *Plant Cell Reports* **23**, 705–709.

Hu, X.R., Yang, A.F., Zhang, K.W., Wang, J. and Zhang, J.R. (2006) Optimization of *in vitro* multiple shoot clump

induction and plantlet regeneration of Kentucky bluegrass (*Poa pratensis*). *Plant Cell, Tissue and Organ Culture* **84**, 89–98.

Huff, D.R. (2003) Kentucky bluegrass. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 27–38.

Inokuma, C., Sugiura, K., Cho, C., Okawara, R. and Kaneko, S. (1996) Plant regeneration from protoplasts of Japanese lawngrass. *Plant Cell Reports* **15**, 737–741.

Inokuma, C., Sugiura, K., Imaizumi, N. and Cho, C. (1998) Transgenic Japanese lawngrass (*Zoysia japonica* Steud.) plants regenerated from protoplasts. *Plant Cell Reports* **17**, 334–338.

Irvin, J.D. (1983) Pokeweed antiviral protein. *Pharmacology and Therapeutics* **21**, 371–387.

Irvin, J.D., Kelly, T. and Robertus, J.D. (1980) Purification and properties of a second antiviral protein from *Phytolacca americana* which inactivates eukaryotic ribosomes. *Archives of Biochemistry and Biophysics* **200**, 418–421.

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kakubari, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2001) Regulation of drought tolerance by gene manipulation of *9-cis-epoxycarotenoid dioxygenase*, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant Journal* **27**, 325–333.

Iuchi, S., Kobayashi, M., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000) A stress-inducible gene for *9-cis-epoxycarotenoid dioxygenase* involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. *Plant Physiology* **123**, 553–562.

Jaglo, K.R., Kleff, S., Amundsen, K.L., Zhang, X., Haake, V., Zhang, J.Z., Deits, T. and Thomashow, M.F. (2001) Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. *Plant Physiology* **127**, 910–917.

James, V.A., Altpeter, F. and Positano, M.V. (2004) Stress-inducible over-expression of transcription factor DREB1A in bahiagrass (*Paspalum notatum* Flugge). *Plant Biology 2004 Abstracts*. American Society of Plant Biologists, Rockville, pp. 68.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) Gus fusions—beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO Journal* **6**, 3901–3907.

Jensen, C.S., Salchert, K., Gao, C., Andersen, C., Didion, T. and Nielsen, K.K. (2004) Floral inhibition in red fescue (*Festuca rubra* L.) through expression of a heterologous flowering repressor from *Lolium*. *Molecular Breeding* **13**, 37–48.

Jensen, C.S., Salchert, K. and Nielsen, K.K. (2001) A TERMINAL FLOWER1-like gene from perennial ryegrass involved in floral transition and axillary meristem identity. *Plant Physiology* **125**, 1517–1528.

Johnson, P.G., Larson, S.R., Anderton, A.L., Patterson, J.T., Cattani, D.J. and Nelson, E.K. (2006) Pollen-mediated gene flow from Kentucky bluegrass under cultivated field conditions. *Crop Science* **46**, 1990–1997.

Jolles, P. and Jolles, J. (1984) What is new in lysozyme research? *Molecular and Cellular Biochemistry* **63**, 165–189.

Kasperbauer, M.J., Buckner, R.C. and Springer, W.D. (1980) Haploid plants by anther-panicle culture of tall fescue. *Crop Science* **20**, 103–107.

Kasperbauer, M.J. and Eizenga, G.C. (1985) Tall fescue doubled haploids via tissue culture and plant regeneration. *Crop Science* **25**, 1091–1095.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1999) Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnology* **17**, 287–291.

Kasuga, M., Miura, S., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2004) A combination of the *Arabidopsis* DREB1A gene and stress-inducible *rd29A* promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. *Plant and Cell Physiology* **45**, 346–350.

Kawakami, A. and Yoshida, M. (2002) Molecular characterization of sucrose: sucrose 1-fructosyltransferase and sucrose: fructan 6-fructosyltransferase associated with fructan accumulation in winter wheat during cold hardening. *Bioscience, Biotechnology and Biochemistry* **66**, 2297–2305.

Ke, S.Q. and Lee, C.W. (1996) Plant regeneration in Kentucky bluegrass (*Poa pratensis* L) via coleoptile tissue cultures. *Plant Cell Reports* **15**, 882–887.

Konstantinova, T., Parvanova, D., Atanassov, A. and Djilianov, D. (2002) Freezing tolerant tobacco transformed to accumulate osmoprotectants. *Plant Science* **163**, 157–164.

Krans, J.V. (1981) Cell cultures of turfgrasses. In: Sheard, R.W. (ed.) *Proceedings of the 4th International Turfgrass Research Conference*. International Turfgrass Society, Guelph, Ontario, Canada, pp. 27–33.

Krans, J.V. and Blanche, F.C.B. (1985) Tissue culture of centipedegrass. In: *Proceedings of the 5th International Turfgrass Research Conference*. Avignon, France, pp. 159–164.

Kriete, G., Niehaus, K., Perlick, A.M., Puhler, A. and Broer, I. (1996) Male sterility in transgenic tobacco plants induced by tapetum-specific deacetylation of the externally applied nontoxic compound *N*-acetyl-L-phosphinothricin. *Plant Journal* **9**, 809–818.

Kuai, B., Dalton, S.J., Bettany, A.J.E. and Morris, P. (1999) Regeneration of fertile transgenic tall fescue plants with a stable highly expressed foreign gene. *Plant Cell, Tissue and Organ Culture* **58**, 149–154.

Kuai, B. and Morris, P. (1995) The physiological-state of suspension-cultured cells affects the expression of the beta-glucuronidase gene following transformation of tall fescue (*Festuca arundinacea*) protoplasts. *Plant Science* **110**, 235–247.

Kuo, Y.J. and Smith, M.A.L. (1993) Plant regeneration from St Augustinegrass immature embryo-derived callus. *Crop Science* **33**, 1394–1396.

Lee, L. (1996) Turfgrass biotechnology. *Plant Science* **115**, 1–8.

Lee, L. and Berg, J.B. (1999) Transgenic plants and method for node segment transformation. US Patent No. 5948956.

Lee, L., Laramore, C.L., Day, P.R. and Tumer, N.E. (1996) Transformation and regeneration of creeping bentgrass (*Agrostis palustris* Huds.) protoplasts. *Crop Science* **36**, 401–406.

Lee, S.H., Lee, D.G., Woo, H.S. and Lee, B.H. (2004) Development of transgenic tall fescue plants from mature seed-derived callus via *Agrobacterium*-mediated transformation. *Asian-Australasian Journal of Animal Sciences* **17**, 1390–1394.

Lee, S.M., Kang, K., Chung, H., Yoo, S.H., Xu, X.M., Lee, S.-B., Cheong, J.-J., Daniell, H. and Kim, M. (2006) Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. *Molecules and Cells* **21**, 401–410.

Leubner-Metzger, G. and Meins Jr, F. (1999) Functions and regulation of plant β-1,3-glucanases (PR-2). In: Datta, S.K. and Muthukrishnan, S. (eds.) *Pathogenesis-Related Proteins in Plants*. CRC, Boca Raton, pp. 49–76.

Levy, Y.Y. and Dean, C. (1998) The transition to flowering. *Plant Cell* **10**, 1973–1989.

Li, L., Li, R., Fei, S. and Qu, R. (2005) *Agrobacterium*-mediated transformation of common bermudagrass (*Cynodon dactylon*). *Plant Cell, Tissue and Organ Culture* **83**, 223–229.

Li, L. and Qu, R. (2002) *In vitro* somatic embryogenesis in turf-type bermudagrass: roles of abscisic acid and gibberellic acid, and occurrence of secondary somatic embryogenesis. *Plant Breeding* **121**, 155–158.

Li, L. and Qu, R. (2004) Development of highly regenerable callus lines and biolistic transformation of turf-type common bermudagrass [*Cynodon dactylon* (L.) Pers.]. *Plant Cell Reports* **22**, 403–407.

Li, Q., Robson, P.R., Bettany, A.J.E., Donnison, I.S., Thomas, H. and Scott, I.M. (2004) Modification of senescence in ryegrass transformed with IPT under the control of a monocot senescence-enhanced promoter. *Plant Cell Reports* **22**, 816–821.

Li, Y., Hagen, G. and Guilfoyle, T.J. (1992) Altered morphology in transgenic tobacco plants that overproduce cytokinins in specific tissues and organs. *Developmental Biology* **153**, 386–395.

Li, R., Bruneau, A.H. and Qu, R. (2006) Improved plant regeneration and in vitro somatic embryogenesis of St Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze]. *Plant Breeding* **125**, 52–56.

Li, R., Bruneau, A.H. and Qu, R. Characterization of morphological somaclonal variants of St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] (Submitted).

Linsmaier, E.M. and Skoog, F. (1965) Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum* **18**, 100–127.

Liu, C.A., Zhong, H., Vargas, J., Penner, D. and Sticklen, M. (1998) Prevention of fungal diseases in transgenic, bialaphos- and glufosinate-resistant creeping bentgrass (*Agrostis palustris*). *Weed Science* **46**, 139–146.

Lowe, K.W. and Conger, B.V. (1979) Root and shoot formation from callus cultures of tall fescue. *Crop Science* **19**, 397–400.

Ludewig, F. and Sonnewald, U. (2000) High CO_2-mediated downregulation of photosynthetic gene transcripts is caused by accelerated leaf senescence rather than sugar accumulation. *FEBS Letters* **479**, 19–24.

Luo, H. and Kausch, A.P. (2002) Application of FLP/*FRT* site-specific DNA recombination system in plants. In: Setlow, J.K. (ed.) *Genetic Engineering, Principles and Methods*. Kluwer/Plenum, New York, Vol. **24**, pp. 1–16.

Luo, H., Hu, Q., Nelson, K., Longo, C. and Kausch, A.P. (2004a) Controlling transgene escape in genetically modified grasses. In: Hopkins, A., Wang, Z.Y., Mian, R., Sledge, M. and Barker, R. (eds.) *Molecular Breeding of Forage and Turf*. Kluwer, Dordrecht, pp. 245–254.

Luo, H., Hu, Q., Nelson, K., Longo, C., Kausch, A.P., Chandlee, J.M., Wipff, J.K. and Fricker, C.R. (2004b) *Agrobacterium tumefaciens*-mediated creeping bentgrass (*Agrostis stolonifera* L.) transformation using phosphinothricin selection results in a high frequency of single-copy transgene integration. *Plant Cell Reports* **22**, 645–652.

Luo, H., Kausch, A.P., Hu, Q., Nelson, K., Wipff, J.K., Fricker, C.R., Owen, T.P., Moreno, M.A., Lee, Y.-Y. and Hodges, T.K. (2005) Controlling transgene escape in GM creeping bentgrass. *Molecular Breeding* **16**, 185–188.

Luo, H., Lee, J.-Y., Hu, Q., Nelson-Vasilchik, K., Eitas, T.K., Lickwar, C., Kausch, A.P., Chandlee, J.M. and Hodges, T.K. (2006) *RTS*, a rice anther-specific gene is required for male fertility and its promoter sequence directs tissue-specific gene expression in different plant species. *Plant Molecular Biology* **62**, 397–408.

Ma, S., Zeng, F., Lan, H., Wu, Z. and Lu, X. (2004) Tissue culture and plantlet regeneration of common centipedegrass. *Communications of Plant Physiology* **40**, 62.

McDonnell, R.E. and Conger, B.V. (1984) Callus induction and plantlet formation from mature embryo explants of Kentucky bluegrass. *Crop Science* **24**, 573–578.

Medford, J., Horgan, R., El-Sawi, Z. and Klee, H.J. (1989) Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. *Plant Cell* **1**, 403–41 3

Meinnel, T., Schmitt, E., Mechulam, Y. and Blanquet, S. (1992) Structural and biochemical characterization of the *Escherichia coli argE* gene product. *Journal of Bacteriology* **174**, 2323–2331.

Meyer, W.A. and Watkins, E. (2003) Tall Fescue. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*, John Wiley & Sons, Hoboken, pp. 107–127.

Mor, A. and Nicolas, P. (1994) The NH2-terminal α-helical domain 1-18 of dermaseptin is responsible for antimicrobial activity. *Journal of Biological Chemistry* **269**, 1934–1939.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473–497.

Nielsen, K. and Boston, R.S. (2001) Ribosome-inactivating proteins: a plant perspective. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 785–816.

Nielsen, K.A. and Knudsen, E. (1993) Regeneration of green plants from embryogenic suspension-cultures of Kentucky blue grass (*Poa pratensis* L). *Journal of Plant Physiology* **141**, 589–595.

Nielsen, K.A., Larsen, E. and Knudsen, E. (1993) Regeneration of protoplast-derived green plants of Kentucky blue grass (*Poa pratensis* L). *Plant Cell Reports* **12**, 537–540.

Nielsen, K.K., Nielsen, J.E., Madrid, S.M. and Mikkelsen, J.D. (1997) Characterization of a new antifungal chitin-binding peptide from sugar beet leaves. *Plant Physiology* **113**, 83–91.

Nishizawa, Y., Kishimoto, N., Saito, A. and Hibi, T. (1993) Sequence variation, differential expression and

chromosomal location of rice chitinase genes. *Molecular and General Genetics* **24**, 1–10.

Nissen-Meyer, J. and Nes, I.F. (1997) Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis and mechanism of action. *Archives of Microbiology* **167**, 67–77.

Oh, S.-J., Song, S.I., Kim, Y.S., Jang, H.-J., Kim, S.Y., Kim, M., Kim, Y.-K., Nahm, B.H. and Kim, J.-K. (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiology* **138**, 341–351.

Ohta, M., Hayashi, Y., Nakashima, A. Hamada, A., Tanaka, A., Nakamura, T. and Hayakawa, T. (2002) Introduction of a Na^+/H^+ antiport gene from *Atriplex gmelini* confers salt tolerance to rice. *FEBS Letters* **532**, 279–282.

Olesen, A., Andersen, S.B. and Due, I.K. (1988) Anther culture response in perennial ryegrass (*Lolium perenne* L). *Plant Breeding* **101**, 60–65.

Olsen, A., Storgaard, M. and Madsen, S. (1996) Suspension culture performance in commercial varieties of perennial ryegrass (*Lolium perenne* L). *Euphytica* **88**, 151–157.

Oliver, M.J., Luo, H., Kausch, A.P. and Collins, H. (2004) Seed-based strategies for transgene containment. In: *Proceedings of 8th International Symposium on the Biosafety of Genetically Modified Organisms*. International Society for Biosafety Research (ISBR), September 26–30, 2004, Montpellier, France, pp. 154–161.

Osusky, M., Osuska, L., Kay, W. and Misra, S. (2005) Genetic modification of potato against microbial diseases: *in vitro* and *in planta* activity of a dermaseptin B1 derivative, MsrA2. *Theoretical and Applied Genetics* **111**, 711–722.

Ow, D.W. (1996) Recombinase-directed chromosome engineering in plants. *Current Opinion in Biotechnology* **7**, 181–186.

Ow, D.W. and Medberry, S.L. (1995) Genome manipulation through site-specific recombination. *Critical Reviews in Plant Science* **14**, 239–261.

Park, S., Li, J., Pittman, J.K., Berkowitz, G.A., Yang, H., Undurraga, S., Morris, Y., Hirschi, K.D. and Gaxiola, R.A. (2005) Up-regulation of a H^+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. *Proceedings of the National Academy of Sciences of the USA* **102**, 18830–18835.

Penmetsa, R.V. and Ha, S.B. (1994) Factors influencing transient gene-expression in electroporated tall fescue protoplasts. *Plant Science* **100**, 171–178.

Pennell, R.L. and Lamb, C. (1997) Programmed cell death in plants. *Plant Cell* **9**, 125–135.

Perezvicente, R., Wen, X.D., Wang, Z.Y., Leduc, N., Sautter, C., Wehrli, E., Potrykus, I. and Spangenberg, G. (1993) Culture of vegetative and floral meristems in ryegrasses—potential targets for microballistic transformation. *Journal of Plant Physiology* **142**, 610–617.

Petrovska, N., Wu, X., Donato, R., Wang, Z.-Y., Ong, E.-K., Jones, E., Forster, J., Emmerling, M., Sidoli, A., O'Hehir, R. and Spangenberg, G. (2004) Transgenic ryegrasses (*Lolium* spp.) with down-regulation of main pollen allergens. *Molecular Breeding* **14**, 489–501.

Pilon-Smits, E.A.H., Ebskamp, M.J.M., Paul, M.J., Jeuken, M.J.W., Weisbeek, P.J. and Smeekens, S.C.M. (1995) Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiology* **107**, 125–130.

Pollock, C.J. and Cairns, A.J. (1991) Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 77–101.

Pontis, H.G. (1989) Fructan and cold stress. *Journal of Plant Physiology* **134**, 148–150.

Pouny, Y., Rapaport, D., Mor, A., Nicolas, P. and Shai, Y. (1992) Interaction of antimicrobial dermaseptin and its fluorescently labeled analogs with phospholipid membranes. *Biochemistry* **31**, 12416–124223.

Qin, X. and Zeevaart, J.A. (1999) The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proceedings of the National Academy of Sciences of the USA* **96**, 15354–15361.

Qin, X. and Zeevaart, J.A. (2002) Overexpression of a *9-cis-epoxycarotenoid dioxygenase* gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiology* **128**, 544–551.

Qu, R. and Chaudhury, A. (2001) Improved young inflorescence culture and regeneration of "Tifway" bermudagrass (*Cynodon transvaalensis* × *C. dactylon*). *International Turfgrass Society Research Journal* **9**, 198–201.

Qu, S.H., Liu, G.F., Zhou, B., Bellizzi, M., Zeng, L., Dai, L.Y., Han, B. and Wang, G.L. (2006) The broad-spectrum blast resistance gene *Pi9* encodes an NBS-LRR protein and is a member of a multigene family in rice. *Genetics* **172**, 1901–1914.

Quaedvlieg, N., Dockx, J., Rook, F., Weisbeek, P. and Smeekens, S. (1995) The homeobox gene *ATH1* of *Arabidopsis* is derepressed in the photomorphogenic mutants *cop1* and *det1*. *Plant Cell* **7**, 117–129.

Ratcliffe, O.J., Amaya, I., Vincent, C.A., Rothstein, S., Carpenter, R., Coen, E.S. and Bradley, D.J. (1998). A common mechanism controls the life cycle and architecture of plants. *Development* **125**, 1609–1615.

Reichman, J.R., Watrud, L.S., Lee, E.H., Burdick, C.A., Bollman, M.A., Storm, M.J., King, G.A. and Mallory-Smith, C. (2006) Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats. *Molecular Ecology* **15**, 4243–4255.

Riordan, T.P. and Browning, S.J. (2003) Buffalograss. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 257–270.

Roylance, J.T., Hill, N.S. and Parrott, W.A. (1994) Detection of somaclonal variation in tissue-culture regenerants of tall fescue. *Crop Science* **34**, 1369–1372.

Ruemmele, B.A. (2003) Colonial bentgrass. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 187–200.

Ruemmele, B.A., Wipff, J., Brilman, L. and Hignight, K. (2003) Fine-leaved *Festuca* species. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 129–174.

Ruf, S., Hermann, M., Berger, I.J., Carrer, H. and Bock, R. (2001) Stable genetic transformation of tomato plastids—high-level foreign protein expression in fruits. *Nature Biotechnology* **19**, 870–875.

Rupp, H.M., Frank, M., Werner, T., Strnad, M. and Schmulling, T. (1999) Increased steady state mRNA levels of the STM and KNAT1 homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. *Plant Journal* **18**, 557–563.

Salehi, H. and Khosh-Khui, M. (2005) Effects of genotype and plant growth regulator on callus induction and plant regeneration in four important turfgrass genera: a comparative study. *In Vitro Cellular and Developmental Biology—Plant* **41**, 157–161.

Sanford, J.C. and Johnston, S.A. (1985) The concept of parasite derived resistance. *Journal of Theoretical Biology* **113**, 395–405.

Saski, C., Lee, S.-B., Fjellheim, S., Guda, C., Jansen, R.K., Luo, H., Tomkins, J., Rognli, O.A., Daniell, H. and Clarke, J.L. (2007) Complete chloroplast genome sequences of *Hordeum vulgare, Sorghum bicolor* and *Agrostis stolonifera*, and comparative analyses with other grass genomes. *Theoretical and Applied Genetics*: DOI 10.1007/s00122-007-0567-4.

Schenk, R.U. and Hildebrandt, A.C. (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant-cell cultures. *Canadian Journal of Botany* **50**, 199–204.

Schnyder, H. (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling—a review. *New Phytologist* **123**, 233–245.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (1999) Molecular responses to drought stress. In: Shinozaki, K. and Yamaguchi-Shinozaki, K. (eds.) *Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants*. R.G. Landes, Austin, pp. 11–28.

Shinozaki, K., Yamaguchi-Shinozaki, K. and Motoaki, S. (2003) Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology* **6**, 410–417.

Shinshi, H., Neuhaus, J.M., Ryals, J. and Meins, F. (1990) Structure of a tobacco endochitinase gene: evidence that different chitinase genes can arise by transposition of sequences encoding a cysteine-rich domain. *Plant Molecular Biology* **14**, 357–368.

Simpson, G.G., Gendall, A.R. and Dean, C. (1999) When to switch to flowering. *Annual Review of Cell and Developmental Biology* **99**, 519–550.

Siobhan, M.J., Alan, C., Cassells, S. and Mohan, J. (2003) Stress and aberrant phenotypes *in vitro* culture. *Plant Cell, Tissue and Organ Culture* **74**, 103–121.

Smigocki, A., Neal Jr, J.W., McCanna, I. and Douglass, L. (1993) Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Molecular Biology* **23**, 325–335.

Spangenberg, G., Wang, Z.Y., Nagel, J. and Potrykus, I. (1994) Protoplast culture and generation of transgenic plants in red fescue (*Festuca rubra* L). *Plant Science* **97**, 83–94.

Spangenberg, G., Wang, Z.Y., Wu, X.L., Nagel, J., Iglesias, V.A. and Potrykus, I. (1995a) Transgenic tall fescue (*Festuca arundinacea*) and red fescue (*F. rubra*) plants from microprojectile bombardment of embryogenic suspension cells. *Journal of Plant Physiology* **145**, 693–701.

Spangenberg, G., Wang, Z.Y., Wu, X.L., Nagel, J. and Potrykus, I. (1995b) Transgenic perennial ryegrass (*Lolium perenne*) plants from microprojectile bombardment of embryogenic suspension cells. *Plant Science* **108**, 209–217.

Srivastava, V. and Ow, D. (2002) Biolistic mediated site-specific integration in rice. *Moecular Breeding* **8**, 345–349.

Stadelmann, F.J., Boller, B., Spangenberg, G., Kolliker, R., Messerli, M., Wang, Z.Y. and Nosberger, J. (1998) Fertility and growth in the field of *Lolium perenne* and *Festuca rubra* plants regenerated from suspension cultured cells and protoplasts. *Plant Breeding* **117**, 37–43.

Stirpe, F. Barbieri, L., Battelli, M.G., Soria, M. and Lappi, D.A. (1992) Ribosome-inactivating proteins from plants: present status and future prospects. *Bio/Technology* **10**, 405–412.

Takahashi, W., Fujimori, M., Miura, Y., Komatsu, T., Nishizawa, Y., Hibi, T. and Takamizo, T. (2005) Increased resistance to crown rust disease in transgenic Italian ryegrass (*Lolium multiflorum* Lam.) expressing the rice chitinase gene. *Plant Cell Reports* **23**, 811–818.

Takamizo, T., Suginobu, K.I. and Ohsugi, R. (1990) Plant regeneration from suspension culture derived protoplasts of tall fescue (*Festuca arundinacea* Schreb.) of a single genotype. *Plant Science* **72**, 125–131.

Taliaferro, C.M. (2003) Bermudagrass (*Cynodon* (L.) Rich). In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 235–256.

Terakawa, T., Sato, T. and Koike, M. (1992) Plant-regeneration from protoplasts isolated from embryogenic suspension-cultures of creeping bentgrass (*Agrostis palustris* Huds.). *Plant Cell Reports* **11**, 457–461.

Theis, T. and Stahl, U. (2004) Antifungal proteins: targets, mechanisms and prospective applications. *Cellular and Molecular Life Sciences* **61**, 437–455.

Thomas, H. and Howarth, C.J. (2000) Five ways to stay green. *Journal of Experimental Botany* **51**, 329–337.

Thompson, C.J., Movva, N.R., Tizard, R., Crameri, R., Davies, J.E., Lauwereys, M. and Botterman, J. (1987) Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. *EMBO Journal* **6**, 2519–2523.

Thorogood, D. (1996) Varietal color of *Lolium perenne* L. turfgrass and its interaction with environmental conditions. *Plant Varieties and Seed* **9**, 15–20.

Thorogood, D. (2003) Perennial ryegrass. In: Casler, M.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons, Hoboken, pp. 75–105.

Tian, W.Z., Yang, M., Ding, Y.Y., Ding, L., Cao, S.Y., Ye, S.Q., Tang, Z.S., Li, L.C., Ling, Z.Z., Zhang, Q. and Hain, R. (2002) Rice transformation with lysozyme gene and bioassay of the transgenic plants and their progenies. In: Tian, W.Z., Li, L.C. and Chen, Y. (eds.) *Future of Agriculture—Gene Transfer Technology*. Hunan Science and Technology, Changsha, pp. 191–197.

Torello, W.A. and Symington, A.G. (1984) Regeneration from perennial ryegrass callus-tissue. *HortScience* **19**, 56–57.

Torello, W.A., Symington, A.G. and Rufner, R. (1984) Callus initiation, plant-regeneration, and evidence of somatic embryogenesis in red fescue. *Crop Science* **24**, 1037–1040.

Toyama, K., Bae, C.H., Kang, J.G., Lim, Y.P., Adachi, T., Riu, K.Z., Song, P.S. and Lee, H.Y. (2003) Production of

herbicide-tolerant zoysiagrass by *Agrobacterium*-mediated transformation. *Molecules and Cells* **16**, 19–27.

Trewavas, A. (2000) Signal perception and transduction. In: Buchanan, B., Gruissem, W. and Jones, R. (eds.) *Biochemistry and Molecular Biology of Plants*. American Society of Plant Biologists, Rockville, pp. 930–987.

Valls, L.A., Hunter, C.P., Rothman, J.H. and Stevens, T.H. (1987) Protein sorting in yeast: the localization determinant of yeast vacuolar carboxypeptidase Y resides in the propeptide. *Cell* **48**, 887–897.

Van Ark, H.F., Zaal, M., Creemers-Molenaar, J. and Van der Valk, P. (1991) Improvement of the tissue culture response of seed-derived callus cultures of *Poa pratensis* L.—effect of gelling agent and abscisic-acid. *Plant Cell, Tissue and Organ Culture* **27**, 275–280.

Van Der Maas, H.M., Dejong, E.R., Rueb, S., Hensgens, L.A.M. and Krens, F.A. (1994) Stable transformation and long-term expression of the *gusA* reporter gene in callus lines of perennial ryegrass (*Lolium perenne* L). *Plant Molecular Biology* **24**, 401–405.

Van Der Valk, P., Proveniers, M.C.G., Pertijs, J.H., Lamers, J.T.W.H., Van Dun, C.M.P. and Smeekens, J.C.M. (2004) Late heading of perennial ryegrass caused by introducing an *Arabidopsis* homeobox gene. *Plant Breeding* **123**, 531–535.

Van Der Valk, P., Ruis, F., Tettelaarschrier, A.M. and Van de Velde, C.M. (1995) Optimizing plant-regeneration from seed-derived callus-cultures of Kentucky bluegrass—the effect of benzyladenine. *Plant Cell, Tissue and Organ Culture* **40**, 101–103.

Van Der Valk, P., Zaal, M. and Creemers-Molenaar, J. (1989) Somatic embryogenesis and plant-regeneration in inflorescence and seed derived callus cultures of *Poa pratensis* L (Kentucky bluegrass). *Plant Cell Reports* **7**, 644–647.

Vergunst, A.C. and Hooykaas, P.J.J. (1998) Cre/*lox*-mediated site-specific integration of *Agrobactetium* T-DNA in *Arabidopsis thaliana* by transient expression of *cre*. *Plant Molecular Biology* **38**, 393–406.

Vergunst, A.C., Jansen, L.E.T. and Hooykaas, P.J.J. (1998) Site-specific integration of *Agrobactetium* T-DNA in *Arabidopsis thaliana* mediated by Cre recombinase. *Nucleic Acids Research* **26**, 2729–2734.

Vigers, A.J., Wiedemann, S., Roberts, W.K., Legrand, M., Selitrennikoff, C.P. and Fritig, B. (1992) Thaumatin-like pathogenesis-related proteins are antifungal. *Plant Science* **83**, 155–161.

Wang, G.R., Binding, H. and Posselt, U.K. (1997) Fertile transgenic plants from direct gene transfer to protoplasts of *Lolium perenne* L and *Lolium multiflorum* Lam. *Journal of Plant Physiology* **151**, 83–90.

Wang, Y., Browning, M., Ruemmele, B.A., Chandlee, J.M. and Kausch, A.P. (2003a) Glufosinate reduced fungal diseases in transgenic glufosinate-resistance bentgrasses (*Agrostis* spp.). *Weed Science* **51**, 130–137.

Wang, Y., Kausch, A.P., Luo, H., Chandlee, J.M., Ruemmele, B.A., Browning, M., Jackson, N. and Goldsmith, M.R. (2003b) Co-transfer and expression of chitinase, glucanase, and *bar* genes in creeping bentgrass for conferring fungal disease resistance. *Plant Science* **165**, 497–506.

Wang, Y., Ruemmele, B.A., Chandlee, J.M., Sullivan, W.M., Knapp, J.E. and Kausch, A.P. (2002) Embryogenic callus induction and plant regeneration media for bentgrasses and annual bluegrass. *In Vitro Cellular and Developmental Biology—Plant* **38**, 460–467.

Wang, Z.Y., Bell, J., Ge, Y.X. and Lehmann, D. (2003a) Inheritance of transgenes in transgenic tall fescue (*Festuca arundinacea* Schreb.). *In Vitro Cellular and Developmental Biology—Plant* **39**, 277–282.

Wang, Z.Y., Bell, J. and Scott, M. (2003b) A vernalization protocol for obtaining progenies from regenerated and transgenic tall fescue plants. *Plant Breeding* **122**, 536–538.

Wang, Z.-Y. and Ge, Y. (2006) Recent advances in genetic transformation of forage and turf grasses. *In Vitro Cellular and Developmental Biology—Plant* **42**, 1–18.

Wang, Z.Y. and Ge, Y.X. (2005a) *Agrobacterium*-mediated high efficiency transformation of tall fescue (*Festuca arundinacea*). *Journal of Plant Physiology* **162**, 103–113.

Wang, Z.Y. and Ge, Y.X. (2005b) Rapid and efficient production of transgenic bermudagrass and creeping bentgrass bypassing the callus formation phase. *Functional Plant Biology* **32**, 769–776.

Wang, Z.-Y., Hopkins, A. and Mian, R. (2001) Forage and turf grass biotechnology. *Critical Reviews in Plant Science* **20**, 573–619.

Wang, Z.Y., Lawrence, R., Hopkins, A., Bell, J. and Scott, M. (2004) Pollen-mediated transgene flow in the wind-pollinated grass species tall fescue (*Festuca arundinacea* Schreb.). *Molecular Breeding* **14**, 47–60.

Wang, Z.Y., Nagel, J., Potrykus, I. and Spangenberg, G. (1993) Plants from cell suspension-derived protoplasts in *Lolium* species. *Plant Science* **94**, 179–193.

Wang, Z.Y., Nagel, J., Iglesias, V.A., Potrykus, I. and Spangenberg, G. (1994) Cryopreservation of embryogenic-cell suspensions in *Festuca* and *Lolium* species. *Plant Science* **103**, 93–106.

Wang, Z.Y., Scott, M., Bell, J., Hopkins, A. and Lehmann, D. (2003c) Field performance of transgenic tall fescue (*Festuca arundinacea* Schreb.) plants and their progenies. *Theoretical and Applied Genetics* **107**, 406–412.

Wang, Z.Y., Takamizo, T., Iglesias, V.A., Osusky, M., Nagel, J., Potrykus, I. and Spangenberg, G. (1992) Transgenic plants of tall fescue (*Festuca arundinacea* Schreb.) obtained by direct gene-transfer to protoplasts. *Bio/Technology* **10**, 691–699.

Warnke, S.E. (2003) Creeping bentgrass. In: Casler, R.D. and Duncan, R.R. (eds.) *Turfgrass Biology, Genetics, and Breeding*, John Wiley & Sons, Hoboken, pp. 175–185.

Watrud, L.S., Lee, E.H., Fairbrother, A., Burdick, C., Reichman, J.R. Bollman, M., Storm, M., King, G. and Van de Water, P.K. (2004) Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proceedings of the National Academy of Sciences of the USA* **101**, 14533–14538.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* **69**, 843–859.

Wipff, J.K. (2002) Gene flow in turf and forage grasses (*Poaceae*). In: *Proceedings of the Scientific methods workshop: ecological and agronomic consequences of gene flow from transgenic crops to wild relatives*. http:/www.biosci.ohio-state.edu/˜asnowlab/Proceedings.pdf.

Wipff, J.K. and Fricker, C. (2001) Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera* L.) in the Willamette valley, Oregon. *International Turfgrass Society Research Journal* **9**, 224–242.

Wu, L. and Jampates, R. (1986) Chromosome number and isoenzyme variation in Kentucky bluegrass cultivars and plants regenerated from tissue culture. *Cytologia* **51**, 125–132.

Wu, Y.-Y., Chen, Q.-J., Chen, M., Chen, J. and Wang, X.-C. (2005) Salt-tolerant transgenic perennial ryegrass (*Lolium perenne* L.) obtained by *Agrobacterium tumefaciens*-mediated transformation of the vacuolar Na^+/H^+ antiporter gene. *Plant Science* **169**, 65–73.

Xiao, L. and Ha, S.B. (1997) Efficient selection and regeneration of creeping bentgrass transformants following particle bombardment. *Plant Cell Reports* **16**, 874–878.

Xu, J.P., Schubert, J. and Altpeter, F. (2001) Dissection of RNA-mediated ryegrass mosaic virus resistance in fertile transgenic perennial ryegrass (*Lolium perenne* L.). *Plant Journal* **26**, 265–274.

Ye, X.D., Wu, X.L., Zhao, H., Frehner, M., Nösberger, J., Potrykus, I. and Spangenberg, G. (2001) Altered fructan accumulation in transgenic *Lolium multiflorum* plants expressing a *Bacillus subtilis sacB* gene. *Plant Cell Reports* **20**, 205–212.

Yu, T.T., Skinner, D.Z., Liang, G.H., Trick, H.N., Huang, B. and Muthukrishnan, S. (2000) *Agrobacterium*-mediated transformation of creeping bentgrass using GFP as a reporter gene. *Hereditas* **133**, 229–233.

Zaghmout, O.M.F. and Torello, W.A. (1988) Enhanced regeneration in long-term callus cultures of red fescue by pretreatment with activated charcoal. *HortScience* **23**, 615–616.

Zaghmout, O.M.F. and Torello, W.A. (1989) Somatic embryogenesis and plant regeneration from suspension cultures of red fescue. *Crop Science* **29**, 815–817.

Zaghmout, O.M. and Torello, W.A. (1990) Isolation and culture of protoplasts from embryogenic suspension-cultures of red fescue (*Festuca rubra* L.). *Plant Cell Reports* **9**, 340–343.

Zaghmout, O.M.F. and Torello, W.A. (1992a) Plant-regeneration from callus and protoplasts of perennial ryegrass (*Lolium perenne* L.). *Journal of Plant Physiology* **140**, 101–105.

Zaghmout, O.M.F. and Torello, W.A. (1992b) Restoration of regeneration potential of long-term cultures of red fescue (*Festuca rubra* L.) by elevated sucrose levels. *Plant Cell Reports* **11**, 142–145.

Zhang, G., Lu, S., Chen, T.A., Funk, C.R. and Meyer, W.A. (2003) Transformation of triploid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* cv. TifEagle) by means of biolistic bombardment. *Plant Cell Reorts* **21**, 860–864.

Zhang, H.X. and Blumwald, E. (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnology* **19**, 765–768.

Zhang, H.X., Hodson, J.N., Williams, J.P. and Blumwald, E. (2001) Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proceedings of the National Academy of Sciences of the USA* **98**, 12832–12836.

Zhong, H., Bolyard, M.G., Srinivasan, C. and Sticklen, M.B. (1993) Transgenic plants of turfgrass (*Agrostis palustris* Huds.) from microprojectile bombardment of embryogenic callus. *Plant Cell Reports* **13**, 1–6.

Zhong, H., Srinivasan, C. and Sticklen, M.B. (1991) Plant regeneration via somatic embryogenesis in creeping bentgrass (*Agrostis palustris* Huds.). *Plant Cell Reports* **10**, 453–456.

Zhu, J.-K. (2000) Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiology* **124**, 941–948.

FURTHER READING

Council for Agricultural Science and Technology Special Publication 25 (2004) *Biotechnology-Derived Perennial Turf and Forage Grasses: Criteria for Evaluation*, at http://www.cast-science.org/cast/src/cast_top.htm

APHIS website. Petition for determination of non-regulated status: roundup ready creeping bentgrass (*Agrostis stolonifera* L.) event ASR368. http://www.aphis.usda.gov/brs/aphisdocs/03_10401p.pdf.

Hu, Q., Nelson, K. and Luo, H. (2006) FLP-mediated site-specific recombination for genome modification in turfgrass. *Biotechnology Letters* **28**, 1793–1804.